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First Named Inventor	John A. Copeland III
Art Unit	1617
Examiner Name	San-ming Hui
Attorney Docket Number	UTSG:240US
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ENCLOSURES (*Check all that apply*)

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February 21, 2006 Michael R. Krawzsenek

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Copland *et al.*

Serial No.: 10/069,744

Filed: July 5, 2002

For: CLINICAL USE OF THIAZOLIDINEDIONES ALONE OR IN CONJUNCTION WITH OTHER AGENTS TO BLOCK OXYTOCIN-MEDIATED ACTIONS SUCH AS UTERINE CONTRACTIONS IN PREMATURE LABOR OR LACTATION

Group Art Unit: 1617

Examiner: San-ming Hui

Atty. Dkt. No.: UTSG:240US

AMENDED APPEAL BRIEF

MAIL STOP APPEAL BRIEF - PATENTS
Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Commissioner:

Appellants hereby submit an original and two copies of this Amended Appeal Brief to the Board of Patent Appeals and Interferences in response to the Office Communication Dated January 18, 2006. Appellants believe that no fees are due with the filing of this Amended Appeal Brief. However, should any fees be due under 37 C.F.R. §§ 1.16 to 1.21, the Commissioner is authorized to deduct any fees from or to Fulbright & Jaworski Deposit Account No. 50-1212/UTSG:240US.

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Exhibit 4: **Fuchs *et al.***
Exhibit 5: **Dullien**
Exhibit 6: ***The Journal of Lipid Research*, Vol. 40, 1177-1184, July 1999**
Exhibit 7: **Final Office Action dated February 23, 2005**

Appendix 3: Related Proceedings Appendix

I. REAL PARTY IN INTEREST

The real party in interest is the assignee, the Board of Regents of the University of Texas System.

II. RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences related to this case.

III. STATUS OF THE CLAIMS

Claims 1-21 were filed with the original application. Claims 1 and 20-21 were canceled in a Response to Restriction Requirement filed January 30, 2004. Claims 2-19 are currently pending in the application. A copy of the pending claims is in the attached Claims Appendix. Claims 2-19 stand rejected and are the subject of this Appeal.

IV. STATUS OF AMENDMENTS

No amendments have been filed after the Final Office Action.

V. SUMMARY OF CLAIMED SUBJECT MATTER

The claimed invention is directed to a method for reducing an oxytocin-mediated action in a subject comprising administering to the subject an amount of thiazolidinedione effective to reduce the oxytocin-mediated action in the subject, wherein the oxytocin-mediated action is induction of labor in a pregnant subject, induction of uterine cramps, induction of milk letdown, or induction of prostaglandin release. *See*, *e.g.*, specification, p. 4, ln. 28 to p. 5, ln. 4; and claims 2-6 as originally filed. In certain embodiments, the claimed method further comprises administering a tocolytic agent. *See*, *e.g.*, specification, p. 4, ln. 17-20; and claim 15 as originally filed.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Claims 15 and 16 stand rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement.

Claims 2-19 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

Claims 2-14 stand rejected under 35 U.S.C. § 103(a), as being unpatentable over Antonucci (Evidence Appendix, Exhibit 1) in view of Hanif (Evidence Appendix, Exhibit 2) and Soloff (Evidence Appendix, Exhibit 3) and Fuchs (Evidence Appendix, Exhibit 4).

Claims 15-19 stand rejected under 35 U.S.C. § 103(a), as being unpatentable over Antonucci in view of Hanif and Soloff and Fuchs, and further in view of Dullien (Evidence Appendix, Exhibit 5).

Claim 2 stands rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-3 of U.S. Patent No. 6,537,566.

VII. ARGUMENT

A. Substantial Evidence is Required to Uphold the Examiner's Position

Findings of fact and conclusions of law by the U.S. Patent and Trademark Office must be made in accordance with the Administrative Procedure Act, 5 U.S.C. § 706(A), (E), 1994. *Dickinson v. Zurko*, 527 U.S. 150, 158 (1999). Moreover, the Federal Circuit has held that findings of fact by the Board of Patent Appeals and Interferences must be supported by "substantial evidence" within the record. *In re Gartside*, 203 F.3d 1305, 1315 (Fed. Cir. 2000). In *In re Gartside*, the Federal Circuit stated that "the 'substantial evidence' standard asks whether a reasonable fact finder could have arrived at the agency's decision." *Id.* at 1312.

Accordingly, an Examiner's position on Appeal must be supported by "substantial evidence" within the record in order to be upheld by the Board of Patent Appeals and Interferences.

B. Claims 15 and 16 Are Enabled Under 35 U.S.C. § 112, First Paragraph

1. The Legal Standard for Enablement

To be enabling within the meaning of 35 U.S.C. § 112, first paragraph, the application must contain a description sufficient to enable one skilled in the art to make and use the claimed invention without unduly extensive experimentation. *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1576 (Fed. Cir. 1984). Furthermore, it is well settled that the Examiner has the initial burden of producing reasons that substantiate a rejection based on lack of enablement. *See In re Marzocchi*, 439 F.2d 220, 224 (C.C.P.A. 1971); *In re Wright*, 999 F.2d 1557, 1561 (Fed. Cir. 1993). The Examiner's burden requires that the Examiner supply a factual basis or scientific principle to reasonably doubt the accuracy of a clear disclosure. *In re Marzocchi*, 439 F.2d at 224.

Enablement under 35 U.S.C. § 112, first paragraph, is not precluded by the necessity for some experimentation such as routine screening. "The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art." *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). In *Wands* the court observed that "[t]he test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed . . . ." *Id.*, quoting *In re Jackson*, 217 USPQ 804, 817 (Bd. App. 1982).

2. Claims 15 and 16 Are Enabled By The Specification

The Examiner asserts that while the specification is enabling for the specific tocolytic agents disclosed in the specification, it does not reasonably provide enablement for any tocolytic agent. Appellants disagree. For convenience, Appellants have reproduced claims 15 and 16 below.

> 15. The method of claim 2, further comprising administering a tocolytic agent.
>
> 16. The method of claim 15, wherein said tocolytic agent comprises a beta-mimetic, magnesium sulfate, a prostaglandin inhibitor, or a calcium-blocking agent.

As long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement is satisfied. *In re Fisher*, 427 F.2d 833, 839 (CCPA 1970). Applicants' specification satisfies this requirement.

The specification teaches that a number of tocolytic agents are known in the art. p. 13, ln. 25-26. The specification also teaches that tocolytic agents.include beta-mimetics, magnesium sulfate, prostaglandin inhibitors, and calcium-blocking agents. Specification at p. 13, ln. 26 to p. 14, ln. 1. Furthermore, the specification identifies numerous specific tocolytic agents including salbutamol, terbutaline, isoxsuprine, ritodrine, fenoterol, magnesium sulfate, indomethacin, aspirin, naproxen, nifedipine, and nicardipine. *Id.* at p. 13, ln. 26 to p. 14, ln. 1.

The use of tocolytic agents, alone, to treat or reduce the "induction of labor in a pregnant animal, induction of uterine cramps, induction of milk letdown, or induction of prostaglandin release" is known in the art. *Id.* at p. 12, ln. 14-23; and p. 13, ln. 22, to p. 14, ln. 24. In addition, thiazolidinedione compounds are currently administered to patients to treat diabetes. *Id.* at p. 4, ln. 8-14. The specification also provides non-limiting examples of how to formulate the co-

administering of thiazolidinedione and a tocolytic agent. *Id.* at p. 25, ln. 1, to p. 27, ln. 9; *see also*, p. 17, ln. 10, to p. 24, ln. 25. It is clear, therefore, that the co-administering of a thiazolidinedione compound in combination with a tocolytic agent would not require undue experimentation because the administration of these types of compounds is practiced in the art.

In fact, the Examiner acknowledges that the specification is enabling for the specific tocolytic agents disclosed in the specification. *See* Action, p. 3. Accordingly, the Examiner admits that the specification discloses not just one, but at least 11 ways in which the skilled artisan can fully practice the claimed invention without undue experimentation. *See In re Fisher*, 427 F.2d at 839. For this reason alone, the rejection of claims 15 and 16 should be reversed.

3. The Examiner Misapplies the Legal Standard For Enablement

The Examiner's enablement rejection is based on improper tests for undue experimentation and unpredictability.

a) An Applicant Is Not Required to Provide an Enabling Disclosure for Technologies Developed After Filing

The Examiner argues that the claims encompass "unknown" and "future known compounds." With regard to these "unknown" and "future known" compounds, the Examiner states: "Hence, those unknown or future known compounds encompassed by claim 1 [*sic*] herein **must** require <u>additional or future research</u> to discover, establish, make and/or verify their usefulness. Therefore... the skilled artisan has to exercise **undue experimentation** to practice the instant invention." Action, p. 9 (emphasis in original). This is not the proper test for undue experimentation.

First, Appellants note that it would not require undue experimentation to make and use the invention, because, as acknowledged by the Examiner, the specification already discloses at least 11 different ways to practice claims 15 and 16. Second, the Examiner's standard for undue

experimentation is legally unfounded. It is well-established law that an applicant need not enable technology developed or invented after the filing date, as such a disclosure would be impossible. *Chiron Corp. v. Genentech, Inc.*, 363 F.3d 1247, 1254 (Fed. Cir. 2004).

b) An Applicant Need Not Prove That the Invention Is Completely Safe

The Examiner also misapplies the law concerning unpredictability in focusing on potential side effects and drug toxicity. *See* Action, p. 6-7 and 10. Testing for the full safety and effectiveness of a particular drug for human use is more properly left to the Food and Drug Administration (FDA). *In re Brana*, 51 F.3d 1560, 1567 (Fed. Cir. 1995). Furthermore, there is nothing in the patent statute or any other statutes that gives the Patent Office the right or the duty to require an applicant to prove that compounds he is claiming, and which he has stated are useful for "pharmaceutical applications," are safe, effective, and reliable for use with humans. *In re Krimmel*, 292 F.2d 948, 954 (C.C.P.A. 1961); *see also* MPEP § 2164.01(c) (noting "The applicant need not demonstrate that the invention is completely safe.").

Moreover, it is not necessary to specify the dosage or method of use if it is known to one skilled in the art that such information could be obtained without undue experimentation. MPEP § 2164.01(c). As discussed above, the co-administering of a thiazolidinedione compound in combination with a tocolytic agent would not require undue experimentation because the administration of these types of compounds is already practiced in the art. *Id.* ("If one skilled in the art, based on knowledge of compounds having similar physiological or biological activity, would be able to discern an appropriate dosage or method of use without undue experimentation, this would be sufficient to satisfy 35 U.S.C. 112, first paragraph.").

c) *The Examiner Misapplies the Holding in Eli Lilly*

Finally, the Examiner asserts that the terms "one beta-mimetic," "at least one prostaglandin inhibitor," and "one-calcium-blocking agent" are "purely functional" terms and that their use is improper. *See* Action, p. 4-5. To support this assertion, the Examiner cites *Regents of the University of California v. Eli Lilly and Co.*, 119 F. 3d 1559 (Fed. Cir. 1997). The Examiner, however, misapplies the requirements set forth in *Eli Lilly*.

Eli Lilly stated, "In claims to genetic material, however, a generic statement such as 'vertebrate insulin cDNA' or 'mammalian insulin cDNA,' without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function." *Eli Lilly*, 119 F.3d 1559, 1568. Claims 15 and 16 of the present invention differ significantly from the claim at issue in *Eli Lilly*. In particular, the current claims are not directed to a genus of tocolytic agents *per se*. Rather, the claims are directed to a method for reducing an oxytocin-mediated action in a subject comprising administering thiazolidinedione. In claim 15, the method further comprises administering a tocolytic agent (*i.e.*, combination therapy). In claim 16, the tocolytic agent is further defined as a beta-mimetic, magnesium sulfate, prostaglandin inhibitor, or calcium blocking agent.

The proper analysis for the presently claimed invention is whether the specification discloses at least one method for reducing an oxytocin-mediated action in a subject comprising administering to the subject an amount of thiazolidinedione effective to reduce the oxytocin-mediated action in the subject, and further comprising administering a tocolytic agent. *See In re Fisher*, 427 F.2d at 839. The Examiner admits that the specification enables at least 11 ways in which the skilled artisan can fully practice the claimed invention without undue experimentation. *See* Action , p. 3. Thus, claims 15 and 16 are enabled.

4. Conclusion

Based on at least the above reasons, the Board should reverse the rejection of claims 15 and 16 under 35 U.S.C. § 112, first paragraph, for lack of enablement.

C. The Claims Are Definite Under 35 U.S.C. § 112, Second Paragraph

The Examiner rejects claims 2-19 as being indefinite. Specifically, the Examiner asserts that the recitations "a subject," "BRL49653," and "a compound related to troglitazone" are indefinite. Appellants traverse this rejection.

A proper evaluation of claims 2-19 under the second paragraph of 35 U.S.C. § 112 requires that the claim be read in light of the specification as interpreted by one of ordinary skill in the art. *North Am. Vaccine, Inc. v. American Cyanamid Co.,* 7 F.3d 1571, 1579, 28 USPQ 2d 1333, 1339 (Fed. Cir. 1993); *In re Moore*, 439 F.2d 1232, 1235 (C.C.P.A. 1971). Furthermore, the law does not require that only immutable or invariant terms be used in claim language. Inventors are encouraged to use concise language, as long as it is reasonably definite in view of the specification. *North Am. Vaccine, Inc.,* 7 F.3d 1571 at 1579.

1. The Term "subject" Is Definite

The Examiner rejects claims 2-19 under 35 U.S.C. § 112, second paragraph, as being indefinite. Specifically, the Examiner contends that claims 2-19 are indefinite because "one of ordinary skill in the art could not ascertain and interpret the metes and bounds of the patent protection desired as to what 'a subject' would be, for example, that the term 'subject' would be a single cell, any biological system, an animal, or a mammal or a human or any subject." The Action, page 11. Appellants traverse this rejection.

The Examiner failed to read the claims in light of the specification as interpreted by one of ordinary skill in the art. This is illustrated by the Examiner's assertion that the term "subject" may be construed as "a single cell." Claim 2 refers to "reducing an oxytocin-mediated action in

a subject" and further defines an oxytocin-mediated action as, for example, "induction of labor in a pregnant subject." A person of ordinary skill in the art would understand that you would not induce labor in a pregnant single cell.

The term "subject" is definite and satisfies all of the requirements of 35 U.S.C. § 112, second paragraph. A person of ordinary skill in the art would understand this term when read in light of the specification. Appellants' specification and claims provide non-limiting examples of "subjects" that are contemplated by the present invention. *See, e.g.*, p. 4, ln. 21-23 ("It is contemplated that the methods described herein can be used for treating mammals, such as humans, as well as other animals."). The term "subject" is clear when read in light of the specification, and the fact that the Examiner prefers other language is not a proper basis for maintaining the present indefinite rejection. *See* MPEP § 2173.01 ("The examiner's focus during examination of claims for compliance with the requirement for definiteness of 35 U.S.C. § 112, second paragraph is whether the claim meets the threshold requirements of clarity and precision, **not whether more suitable language or modes of expression are available.**") (emphasis added).

The rejection of claims 2-19 under 35 U.S.C. § 112, second paragraph, for indefiniteness is improper and should be withdrawn.

2. The Term "BRL49653" Is Definite

The Examiner rejects claim 9 under 35 U.S.C. § 112, second paragraph, for indefiniteness. Specifically, the Action contends that the term "BRL49653" is an abbreviation or trademark/trade name and is therefore indefinite.

Appellants disagree. The term "BRL49653" is definite, and claim 9 satisfies all of the requirements under 35 U.S.C. § 112, second paragraph.

The term "BRL49653" is neither an abbreviation nor a trademark. This term is a synonym recognized by a person of ordinary skill in the art to describe a thiazolidinedione compound with the IUPAC-style chemical name 5-[[4-[2-(methyl-2-pyridinylamino)ethoxy]phenyl] methyl]-2,4-thiazolidinedione, which is also known by the synonym "rosiglitazone" and has the CAS number 122320-73-4. *See* Specification, p. 15, ln. 30, to p. 16, ln. 5; *see also* Edvardsson *et al.*, "Rosiglitazone (BRL49653), a PPAR-selective agonist, causes peroxisome proliferator-like liver effects in obese mice," *The Journal of Lipid Research*, Vol. 40, 1177-1184, July 1999 (Evidence Appendix, Exhibit 6). A person of ordinary skill in the art would therefore understand the meaning of the term "BRL49653" when read in light of the specification. *See* MPEP 2173.01.

Furthermore, despite the Examiner's contention, the use of a trademark or trade name in a claim is accepted practice. In this regard, the MPEP states:

> Names used in trade **are permissible** in patent applications if:
> (A) Their meanings are established by an accompanying definition which is sufficiently precise and definite to be made a part of a claim, or
> (B) In this country, their meanings are well-known and satisfactorily defined in the literature.

MPEP § 608.01(v) (emphasis added). The term "BRL49653" is (1) defined in Appellants' specification, and (2) known by persons skilled in the art. The use of this term in the claim is therefore appropriate under current patent laws.

The rejection of the term "BRL49653" as being indefinite is therefore improper and should be reversed.

3. *The Phrase "A Compound Related to Troglitazone" Is Definite*

The Examiner also rejects claim 9 under 35 U.S.C. § 112, second paragraph, for indefiniteness. Specifically, the Examiner contends that the phrase "a compound related to

troglitazone" is indefinite. Appellants disagree. This phrase is definite, and claim 9 satisfies all of the requirements under 35 U.S.C. § 112, second paragraph.

A person of ordinary skill in the art would under stand the scope of the phrase "a compound related to troglitazone" when read in light of the specification. *See* MPEP § 2173.02. In a non-limiting embodiment, for example, the specification recites: "A compound related to troglitazone is one that is substantially similar to the chemical structure of troglitazone or can be derived from troglitazone." Specification, p. 5, ln. 8-10. One of ordinary skill in the art could therefore ascertain the scope of this phrase in view of the specification. *See* MPEP § 2173.02.

The rejection of the phrase "a compound related to troglitazone" as being indefinite is therefore improper and should be reversed.

D. The Claims Are Patentable Over the Cited References

1. The Legal Standard for Obviousness

It is well settled that "[t]he examiner bears the initial burden of factually supporting any *prima facie* case of obviousness. If the examiner does not produce a *prima facie* case, the applicant is under no obligation to submit evidence of nonobviousness." MPEP § 2142.

To establish a *prima facie* case of obviousness, the Action must show: (1) some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) a reasonable expectation of success; and (3) the prior art reference teaches or suggests all of the claim limitations. *Id.*; *see also In re Vaeck*, 947 F.2d 488, (Fed Cir. 1991). With respect to element (1), "[t]he mere fact that references <u>can</u> be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination." MPEP § 2143.01. If any one of the three elements is missing, an obviousness rejection cannot be maintained.

2. *Claims 2-19 are Not Obvious Over the Cited References*

a) Summary of Rejections

Claims 2-14 stand rejected under 35 U.S.C. § 103(a) as being obvious over Antonucci in view of Hanif, Soloff, and Fuchs. Dependent claims 15-19 are further rejected as being obvious over the same references and further in view of Dullien. The Action contends that Antonucci discloses the use of thiazolidinedione compounds such as troglitazone for the treatment of normal pregnant women or non-diabetic pregnant women due to insulin resistance and/or related risks. It is admitted by the Action, however, that Antonucci fails to teach or suggest using thiazolidinedione compounds in Appellants' claimed method for reducing oxytocin-mediated actions such as induction of labor, induction of uterine cramps, induction of milk letdown, or the induction of prostaglandin release.

In an attempt to supplement the deficient teachings of Antonucci, the Action cites to Hanif, Soloff, and Fuchs and contends that it would have been obvious to use the thiazolidinedione compounds in Antonucci for Appellants' claimed method. Specifically, the Action contends that Hanif teaches that "apparently, in the adipocyte, oxytocin acts via the same receptor as is present in uterine and breast smooth muscle and the metabolic actions of oxytocin are due to mechanisms in common … with ones involved in the action of insulin." Final Office Action dated February 23, 2005 at p. 14 (Evidence Appendix, Exhibit 7). As for Soloff and Fuchs, the Action contends that these references disclose that oxytocin is involved with smooth muscle contraction during birth, milk letdown during lactation and prostaglandin release from endometrium/deciduas and the anmnion.

With respect to claims 15-19, the Action admits that Antonucci, Hanif, Soloff, and Fuchs fail to teach the employment of a tocolytic agent in combination with thiazolidinedione in methods for reducing oxytocin-mediated actions (such as milk letdown) in a pregnant mammal.

In an attempt to supplement the deficient teachings of these references, the Action cites to Dullien and contends that it would have been obvious to use a tocolytic agent in combination with thiazolidinedione.

Appellants traverse this rejection. Claims 2-19 are not rendered obvious over the cited references.

b) There Is No Motivation to Combine the References

In order to establish a *prima facie* case of obviousness, the Action must show that there is a motivation to modify or combine the teachings of Antonucci with those of Hanif, Soloff, and Fuchs. There is no motivation to combine the cited references, and the Action has provided no evidence to the contrary.

Antonucci appears to focus on problems concerning insulin resistance and diabetes. As admitted by the Action, this reference is not concerned with oxytocin-mediated actions—much less reducing the "induction of labor in a pregnant animal, induction of uterine cramps, induction of milk letdown, or induction of prostaglandin release." Further, a person of ordinary skill in the art would recognize that diabetes is a vastly different disease from reducing the oxytocin-mediated actions claimed by Appellants.

Hanif's teachings are also deficient. Hanif appears concerned with determining "the effects of oxytocin on glucose transport, glucose oxidation, and lipogenesis" and comparing "these effects with the actions of insulin." Hanif, Summary. This reference, in fact, does not appear to mention or suggest thiazolidinedione—much less the use of thiazolidinedione for reducing an oxytocin-mediated action claimed by Appellants.

The Examiner's assertion that Hanif teaches that "in the adipocyte, oxytocin acts via the same receptor as is present in uterine and breast smooth muscle and the metabolic actions of oxytocin are due to mechanisms in common (chem. Mediators, phosphorylation-

dephosphorylation reactions) with one involved in the action of insulin" fails to establish a motivation to combine Hanif with Antonucci. Final Office Action at p. 14. The possibility that Hanif discloses that some "metabolic actions" of oxytocin are due to mechanisms in common with insulin provides little, if any, suggestion that such teachings could be used for reducing the oxytocin-mediated actions claimed by Appellants. This is particularly true in view of Hanif's teachings that:

- "the insulin-like activity of oxytocin is due to oxytocin binding to its own receptor and ***not to the insulin receptor***." Hanif, p. 381, col. 1 (emphasis added);
- "*N*-carbamoyl-*O*-methyl oxytocin, a specific oxytocin antagonist, inhibits oxytocin-stimulated lipogenesis ***without affecting the insulin response***." Hanif, p. 381, col. 1 (emphasis added);
- "adipocytes from homozygous diabetes insipidus rats (Brattleboro strain) demonstrate normal insulin-stimulated responses ***while oxytocin is unable to stimulate glucose oxidation***." Hanif, p. 381, col. 1 (emphasis added).

Similar to the deficient teachings of Antonucci and Hanif, the Soloff and Fuchs references also appear to fail to mention or suggest the use of thiazolidinedione. Further, there does not appear to be any suggestion in these references that thiazolidinedione compounds could be used to reduce the oxytocin-mediated actions claimed by Appellants. It appears that the Examiner is relying on hindsight to find a motivation to combine these references. The use of hindsight, however, is not appropriate to establish a motivation to combine. *See W.L. Gore Assoc., Inc. v. Garlock, Inc.*, 721 F.2d 1540 (Fed. Cir. 1983); MPEP § 2143.01 ("The mere fact that references <u>can</u> be combined or modified does not render the resultant combination obvious unless the prior art also **suggests the desirability** of the combination.") (emphasis added).

Because of the lack of motivation to combine, a *prima facie* case of obviousness has not been established. The obviousness rejection should therefore be reversed.

c) The References Do Not Provide a Reasonable Expectation of Success

A second element necessary to establish a *prima facie* case of obviousness requires a showing of a reasonable expectation of success that combining the teachings of Antonucci with the teachings of Hanif, Soloff, and Fuchs would work. This has not been shown by the Examiner.

Appellants' specification, in non-limiting embodiments, provides data that shows the reduction of oxytocin-mediated activities by a thiazolidinedione compound. *See, e.g.*, specification, p. 29, ln. 15, to p. 31, ln. 12. These data show a reduction of prostaglandin E_2 "release by oxytocin when 5 μg/ml of troglitazone was added to a culture of primary human myometrial cells minutes prior to oxytocin treatment." *Id.* at page 29, line 25. Additional data show a dose of 10 μg/ml of troglitazone (a thiazolidinedione compound) inhibited 10 nM oxytocin-induced contractions in a strip of term myometrial tissue obtained from C-section deliveries. *Id.* at page 30, line 30.

The Antonucci reference, by contrast, does not appear to provide any data showing that a thiazolidinedione compound can be used to reduce the oxytocin-mediated actions claimed by Appellants. Rather, this reference appears to be concerned with the treatment of diabetes—not the induction of labor in a pregnant subject, induction of uterine cramps, induction of milk letdown, nor induction of prostaglandin release. Based on this evidence, or lack thereof, it cannot be reasonably contended that there is a reasonable expectation of success to modify Antonucci to employ Appellants' invention. There is simply no data in this reference to support such an assertion.

The secondary references, Hanif, Soloff, and Fuchs similarly fail to provide a reasonable expectation of success. These references lack any data suggesting that the use of a thiazolidinedione compound could be used to reduce the oxytocin-mediated actions claimed by Appellants. The secondary references, in fact, appear to fail to mention thiazolidinedione compounds altogether. Furthermore, while Hanif may disclose that ***some*** "metabolic actions" of oxytocin are due to mechanisms in common with insulin, it also notes several distinctions between the mechanisms, including the facts that oxytocin does not bind the insulin receptor (Hanif, p. 381, col. 1); a specific oxytocin antagonist ***did not*** affect the insulin response (Hanif, p. 381, col. 1); and diabetic rat adipocytes demonstrated normal insulin-stimulated responses while oxytocin is unable to stimulate glucose oxidation (Hanif, p. 381, col. 1). Such distinctions between the actions of oxytocin and insulin would certainly not provide a reasonable expectation of success in achieving Appellants' claimed invention.

d) Conclusion

Based on at least the above arguments and evidence, the Examiner failed to establish a *prima facie* case of obviousness. The present obviousness rejection should therefore be reversed.

3. Dependent Claim 7 is Separately Patentable

Dependent claim 7 is separately patentably from independent claim 2. Claim 7 includes the limitation of "wherein the subject is a mammal." The cited references do not appear to disclose a method for reducing an oxytocin-mediated action in a mammal comprising administering an amount of thiazolidinedione. In fact, the Action admits that Antonucci *et al.* fails to disclose "the employment of the particular thiazolidedione compounds, in methods for reducing oxytocin-mediated action in a pregnant mammal." Further, and as noted above, none of the secondary references appear to mention or suggest thiazolidedione compounds—much less the use of such compounds in Appellants' claimed process.

Because the cited references fail to disclose the use of thiazolidedione for reducing an oxytocin-mediated action in a mammal, a *prima facie* case of obviousness for claim 7 has not been established. *See* MPEP § 2142.

4. Dependent Claims 15-19 are Separately Patentable

Dependent claims 15-19 are separately patentable from independent claim 2. Claim 15 includes the additional limitation "further comprising administering a tocolytic agent." Claims 16-18 are directed towards specific tocolytic agents, and claim 19 is directed towards administration of the tocolytic agent and thiazolidinedione simultaneously.

There is no motivation to combine the teachings of Dullien with those of Antonucci, Hanif, Soloff, or Fuchs. Of the four references cited by the action, only one (Antonucci) appears to discuss thiazolidinedione compounds—and that is in the context of insulin resistance and diabetes and **not** Applicants' claimed method of reducing an oxytocin-mediated action in a subject. Additionally, Dullien is the only cited reference that appears to disclose the use of tocolytic agents.

Neither Antonucci nor Dullien, for example, suggest using the combination of a thiazolidinedione compound with a tocolytic agent to perform Applicants' claimed method, and the Action has failed to present any evidence to the contrary. The mere fact that their teachings can be combined is insufficient to establish a motivation to combine. There must be a suggestion of the desirability of the combination, evidence which the Action has failed to produce. MPEP § 2143.01 ("[t]he mere fact that references <u>can</u> be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination."). In fact, there does not appear to be any desirability of combining Dullien with Antonucci because these references concern different therapies. Stated another way, a person of

ordinary skill would not look to the art of insulin resistance and diabetes (Antonucci) to solve the problems associated with pre-term labor (Dullien), and *vice versa*.

Because there is no motivation to combine Dullien with the other cited references, a *prima facie* case of obviousness for claims 15-19 has not been established. *See* MPEP § 2142.

E. The Double Patenting Rejection

Claim 2 is rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-3 of U.S. Patent 6,537,566. The Examiner notes that this rejection can be overcome by filing a terminal disclaimer pursuant to 37 C.F.R. § 1.321(c). Appellants note that an appropriate Terminal Disclaimer will be filed upon the indication of allowable subject matter. Until then, Appellants do not believe that it is necessary to prepare a Terminal Disclaimer and pay the corresponding fee.

VIII. CONCLUSION

It is respectfully submitted, in light of the above, that all claims are in condition for allowance. Appellants, therefore, requests that the Board overturn each of the pending grounds for rejection.

Please date stamp and return the enclosed postcard to evidence receipt of this document.

Respectfully submitted,

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Date: February 21, 2006

APPENDIX 1
Claims Appendix

2. A method for reducing an oxytocin-mediated action in a subject comprising administering to the subject an amount of thiazolidinedione effective to reduce the oxytocin-mediated action in the subject, wherein the oxytocin-mediated action is induction of labor in a pregnant subject, induction of uterine cramps, induction of milk letdown, or induction of prostaglandin release.

3. The method of claim 2, wherein the oxytocin-mediated action is induction of labor in a pregnant subject.

4. The method of claim 2, wherein the oxytocin-mediated action is induction of uterine cramps.

5. The method of claim 2, wherein the oxytocin-mediated action is induction of milk letdown.

6. The method of claim 2, wherein the oxytocin-mediated action is induction of prostaglandin release.

7. The method of claim 2, wherein the subject is a mammal.

8. The method of claim 2, wherein the thiazolidinedione is troglitazone.

9. The method of claim 2, wherein the thiazolidinedione is pioglitazone, BRL49653, or a compound related to troglitazone.

10. The method of claim 2, wherein the thiazolidinedione is dispersed in a pharmacologically acceptable form.

11. The method of claim 10, wherein said thiazolidinedione is administered locally.

12. The method of claim 10, wherein said thiazolidinedione is administered parenterally.

13. The method of claim 12, wherein said thiazolidinedione is administered intravenously.

14. The method of claim 11, wherein the thiazolidinedione is administered intravaginally.

15. The method of claim 2, further comprising administering a tocolytic agent.

16. The method of claim 15, wherein said tocolytic agent comprises a beta-mimetic, magnesium sulfate, a prostaglandin inhibitor, or a calcium-blocking agent.

17. The method of claim 16, wherein the prostaglandin inhibitor is indomethacin.

18. The method of claim 17, wherein the calcium-blocking agent is nifedipine.

19. The method of claim 15, wherein the tocolytic agent and thiazolidinedione are administered simultaneously.

APPENDIX 2
Evidence Appendix

17

Endocrine Control of Parturition

MELVYN S. SOLOFF

1. *Problems in Understanding Basic Mechanisms of Parturition*

In their introduction to the previous edition, Thorburn *et al.* (1977) stated: "We now recognize that in late pregnancy a train of events is initiated that ultimately results in the delivery of the fetus. However, we still do not know exactly how and where the train starts, or exactly how it exerts its ultimate action on the myometrial cell." Little has changed in the last decade to increase our understanding of these events. Although the initiation of parturition is generally understood, the precise trigger for labor is still unknown. In addition, labor is complicated by different mechanisms in different species. For example, the onset of labor in rats and rabbits is rapid: uterine contractions become intense immediately before delivery, and the newborn are expelled rapidly. In humans, monkeys, and guinea pigs, labor develops slowly and is protracted. Schofield (1968) suggested that in species with a large fetus relative to the mother, a more protracted delivery may be an advantage. In the human and monkey, uterine motility evolves gradually during the last trimester of pregnancy, and actual labor often precedes delivery by many hours. It is possible that different mechanisms are at play in rapid-onset and protracted-onset types of labor.

Generally, attempts to explain the initiation of labor have focused on labor-associated changes in the circulating concentrations of hormones in both fetus and mother. They illuminated the importance of the fetal production of cortisol in the initiation of labor in sheep, cows, and goats. In other species, however, the important factor may be the changes in receptor levels for specific hormones. Examples of both agonist and receptor regulation of myometrial activity are cited in Sections 3 and 4.

Many investigators believe that the maintenance of the pregnant uterus results from a balance between factors promoting and inhibiting the termination of pregnancy, such as the balance between oxytocic agents and progesterone. Reduction in progesterone reduces the quiescent state of the uterine musculature, resulting in labor contractions.

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In the rat and rabbit, the concentration of progesterone decreases in the maternal peripheral plasma before labor, and delivery is usually preceded by an increase in the concentration of circulating estrogen. Gestation can be abruptly terminated by administration of agents that block the synthesis of progesterone. The administration of progesterone prevents the effects of these agents and prolongs gestation in otherwise untreated animals.

Withdrawal of progesterone from the systemic maternal circulation is not required in primates and guinea pigs for the initiation of parturition. In these species, maternal plasma progesterone levels remain elevated until delivery of the placenta. Labor cannot be prolonged by administration of progesterone. Therefore, although progesterone withdrawal may be important in some species, it does not readily explain the onset of labor in others.

To add to the complexity, parturition may be triggered by several initiating agents. Triggering of the expulsion of the newborn requires the interplay of multiple endocrine factors that prepare uterine cells to respond to the appropriate uterotonic stimuli. In some cases, it is difficult to distinguish between the action of an agent in the development of the response and in the response itself.

Despite the present lack of understanding of the precise sequence of molecular events leading to labor contractions, however, there are some features of parturition common to all species, suggesting that mechanisms involved in labor initiation may be basically the same. These include roles for estrogens and prostaglandins. In addition, the sensitivity of the uterine musculature to oxytocin is greatest at the time of labor in all species studied, suggesting an important role for this peptide. These common features of parturition are discussed in the sections that follow.

2. *Preparation of the Uterus for Labor: Estrogen*

Estrogens are vital for the preparation of the uterus for parturition, but it is not clear whether increases in blood estrogen levels at the end of gestation serve as a trigger for the initiation of labor. Estrogen treatment results in hyperplasia and hypertrophy of uterine cells and in stimulation of the synthesis of contractile proteins, metabolic enzymes, and ATP (Marshall, 1974). Estrogens also contribute to the sharp rise in glycogen content of the rat myometrium just before labor (Chew and Rinard, 1979). In addition, estrogens elicit heterologous up-regulation of hormone and growth factor receptors in the myometrium. These include receptors for oxytocin, α-adrenergic agonists, serotonin (Ichida, 1983), angiotensin II (Schirar *et al.*, 1980), and epidermal growth factor (Mukku and Stancel, 1985). Estrogens also stimulate the biosynthesis of prostaglandins; they regulate myometrial levels of calcium and modify the phosphorylation of specific proteins in the myometrium at the time of labor, so that putative second messenger pathways also are stimulated. Changes in the phosphorylation of specific myometrial proteins at the time of labor in the rat (Fig. 1) can be mimicked in nonpregnant animals by administration of estrogen (Joseph *et al.*, 1982). Estrogen also induces oxytocin-inhibited (Ca^{2+}, Mg^{2+})-ATPase activity in rat myometrium (Soloff and Sweet, 1982).

Estrogens regulate membrane components involved in the permeability of Na^+, K^+, and Cl^-, which are responsible for the resting membrane potential and electrical excitability of myometrial cells (Marshall, 1974). Injection of poly(A)+ RNA from estrogen-treated rat uteri into frog oocytes resulted in the expression of a very slow-activating votage-dependent postassium current (Boyle *et al.*, 1987). The mRNA expressing the channel was rapidly and reversibly induced by estrogen, as indicated by its appearance and disappearance during the estrous cycle, its reappearance at the end of pregnancy when the uterus is estrogen dominated, and its induction by estrogen treatment in ovariectomized rats. Garfield and colleagues (1980) have shown that estrogens appear to promote synchronous uterine contractility by stimulating

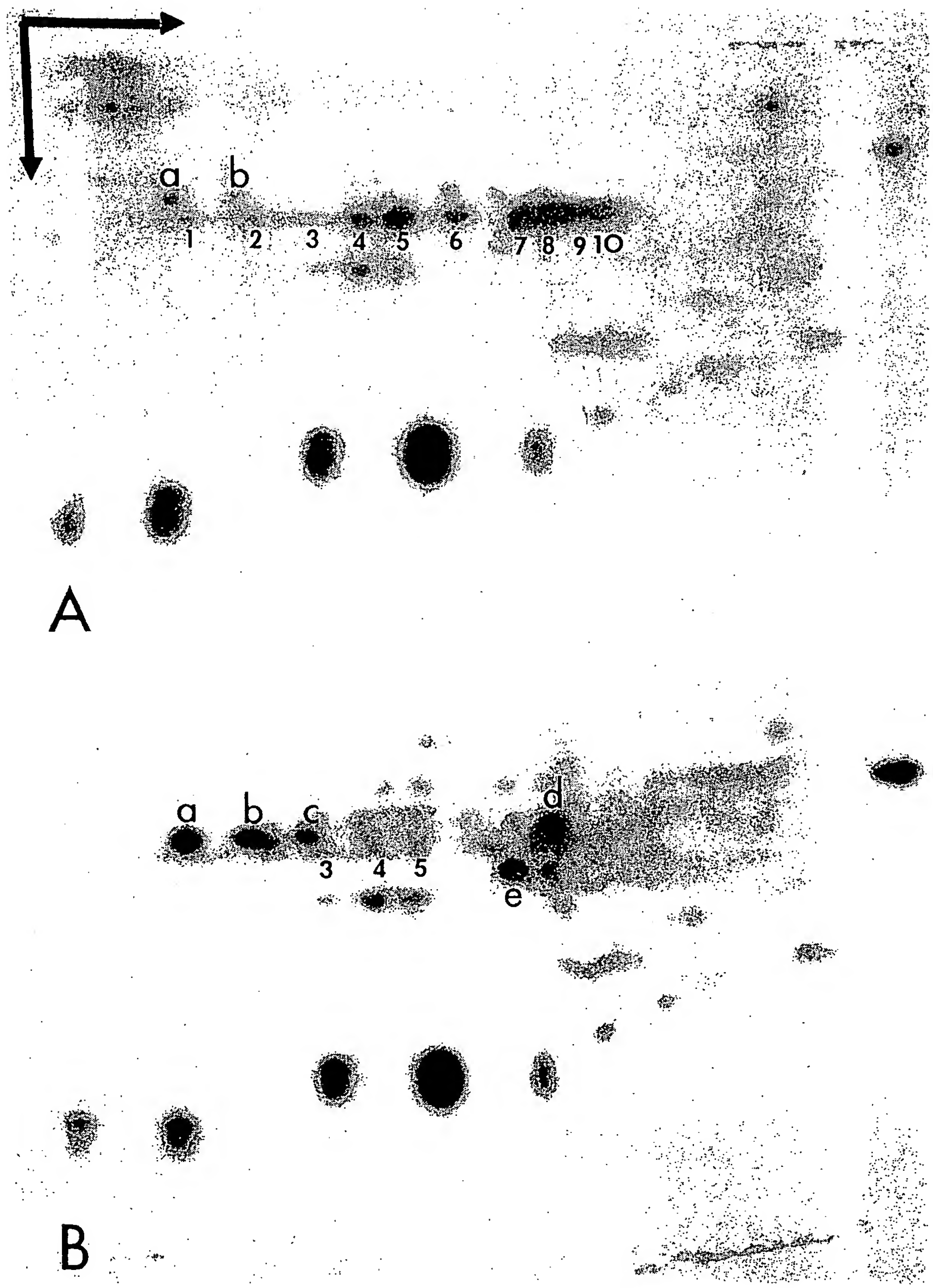

Figure 1. Changes in the phosphorylation of specific rat myometrial proteins during labor, as demonstrated by two-dimensional gel electrophoresis. (A) Day 19 of pregnancy. (B) Labor. Myometrial minces were incubated with $^{32}P_i$ for 60 min, and the proteins were analyzed for incorporation of radioactivity after electrophoresis by fluorography. Labor-specific phosphoproteins are indicated by a–e. From Joseph *et al.* (1982).

the formation of gap junctions between adjacent myometrial cells. The estrogen up-regulation of progesterone receptors (Vu Hai *et al.*, 1977) may explain why many of the physiological effects of progesterone are dependent on previous exposure to estrogen.

Estrogens also are involved in cervical maturation: collagen breakdown in the cervix is caused by increasing collagenase synthesis and activation. Overall, estrogen actions on the myometrium include metabolic, membrane, and structural changes that promote spontaneous contractility and enhance myometrial responsiveness to many agonists.

As an example of the importance of estrogens in primates, suppression of adrenal estrogen precursor formation in rhesus monkeys prolonged pregnancy (Novy, 1983). Treatment of monkeys for about the last 65 days of pregnancy (gestation length about 167 days) with dexamethasone caused adrenal atrophy, reduced basal levels of maternal estradiol (but not progesterone), and abolished the prepartum estrogen surge. More than 70% of the fetuses were born after day 175 of gestation. However, large doses of estradiol benzoate administered systemically to pregnant rhesus monkeys did not cause premature labor unless the fetuses were dead (Walsh *et al.*, 1979a). Such findings indicate that estrogens are important in primates for the development of the ability of the uterus to contract at the end of gestation, but they do not play a role in triggering labor. In other species, however, the administration of estrogen near the end of gestation is a very effective means of inducing premature labor (see Thorburn and Challis, 1979, for references), perhaps by estrogen-induced increases in the concentration of oxytocin receptors and the number of myometrial gap junctions.

2.1. *Possible Regulation of Estrogen Action at the Receptor Level*

The concentrations of estrogen receptors in both the nuclear fraction and the cytosol of rat myometrium increased abruptly, were maximal near the time of labor, and fell abruptly after parturition (Fig. 2). Because estrogen up-regulates its own receptor concentration (Pavlik and Coulson, 1976), it is likely that the occurrence of estrogen dominance in the rat before parturition is enhanced by increases in both circulating estrogen concentrations and receptor number. In contrast to the rat, no significant labor-related changes in estrogen receptor concentrations appeared in human uteri (Giannopoulos *et al.*, 1980). In humans increased plasma estrogen concentrations may be sufficient to bring about estrogen dominance at the end of gestation.

2.2. *The Role of Estrogens in Humans*

The importance of estrogens in human pregnancy is controversial. Pinto *et al.* (1967), using a double-blind design, gave intravenous infusions of either estradiol or vehicle to equal numbers of women at term. Those given estradiol went into spontaneous labor significantly sooner or required significantly lower doses of intravenous oxytocin for labor induction. Estrogen administration seemed to facilitate the onset of labor (Järvinen *et al.*, 1965). However, Klopper and Dennis (1962) found no effect of estrogen on the length of labor.

2.3. *Identification of Active Estrogens in Humans*

Darne and co-workers (1987) suggested that estriol is more active in the human than is usually assumed. Estriol was considered to be a weak or impeded estrogen because there was no sustained uterine growth following a single injection of estriol compared with a similar dose of estradiol. Repeated doses of estriol, rather than a single injection, produced a full uterine

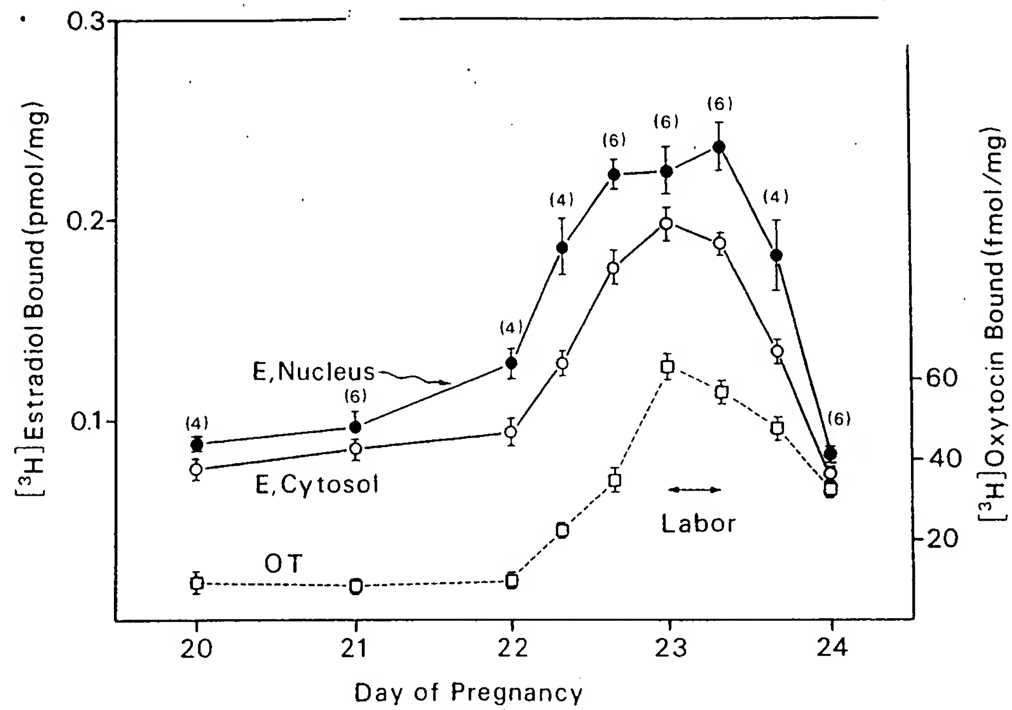

Figure 2. Increases in the concentration of estrogen receptors in the nuclear (●) and cytosol (○) fractions of rat myometrium preceding labor. Each point is the mean ± S.E., with the number of rats shown in parentheses. Oxytocin (OT) receptor concentrations in the myometrium are also shown. From Alexandrova and Soloff (1980a).

growth response similar to that to estradiol (Clark and Markaverich, 1984). The apparent weakness of estriol could be explained partly by its shorter half-life in plasma and its shorter occupancy of uterine estrogen receptors.

Both human endometrium and myometrium have the ability to accumulate tritium-labeled estriol and estradiol with equal facility following continuous infusion of subphysiological doses before hysterectomy (Wiegerinck *et al.*, 1983). Estriol is as effective as estradiol in stimulating $PGF_{2\alpha}$ production by human endometrial cells in culture (Schatz *et al.*, 1984). Darne and co-workers (1987) found that the ratio of estriol to progesterone in the saliva of women was maximal near the time of labor. These workers suggested that a fine balance is maintained between free progesterone and free estriol and estradiol throughout pregnancy until a few weeks before delivery. The surge in the estriol : progesterone ratio secondary to the rise in estriol levels than overcomes the myometrial stabilizing effect of progesterone and provokes the changes in the uterus (such as an increase in the number of oxytocin receptors and gap junctions, an increase in the synthesis and release of prostaglandins, and an increase in the levels of free intracellular calcium) that are necessary for normal labor.

2.4. *Control of Estrogen Levels*

The endocrine mechanisms regulating parturition in ruminants or laboratory animals and primates differ in several respects. The primate fetus contributes estrogens by adrenal production of estrogen precursors (i.e., androstenedione and dehydroepiandrosterone sulfate), which are converted to estrogens by the placenta (Diczfalusy, 1974; Walsh *et al.*, 1980). The fetus thus provides a mechanism for the endocrine control of parturition.

It ha[illegible]t been possible to demonstrate that the hun[illegible] fetus is involved in the initiation of labor. The human fetal pituitary–adrenal axis, however, plays an indirect role by the secretion of estrogen precursors by the fetal zone of the fetal adrenal, thereby increasing the plasma concentration of estrogen in mother and fetus. In women, there is a gradual but steady increase in plasma estradiol-17β and estrone sulfate concentrations from 36 weeks of gestation. In normal rhesus monkeys, estradiol and estrone concentrations increase gradually and significantly in maternal blood during the 7 to 10 days preceding spontaneous vaginal delivery (Novy, 1983). Progesterone does not decrease before parturition and may increase.

The stimulus for the rise in fetal and maternal estrogens before parturition is unknown, but a functioning fetal adrenal in monkeys is required because the prepartum estrogen trends are absent when the fetus is dead or functionally hypophysectomized (Walsh *et al.*, 1979a). In addition, the administration of ACTH, but not other peptides, to the fetus results in augmented fetal dehydroepiandrosterone sulfate, androstenedione, progesterone, and estrogen concentrations (Walsh *et al.*, 1979b). Continuous infusion of ACTH to the fetus also produced elevated estrogen levels in the amniotic fluid (Novy, 1983). It remains to be shown in rhesus monkeys, however, that levels of ACTH rise in fetal plasma before parturition.

3. *Uterine Quiescence*

3.1. *Progesterone*

The inhibitory effect of progesterone on myometrial activity has been referred to as "progesterone block" (Csapo, 1956). It is characterized by a dose-dependent suppression of the amplitude of myometrial contractions, reduction in the rate of rise of pressure during intrauterine pressure cycles, and loss of reactivity to oxytocic agents (Csapo 1956; Schofield 1957; Csapo and Takeda, 1965). Marshall (1959) found that progesterone treatment of estrogen-primed ovariectomized rats increased the membrane potential in single uterine fibers and caused the dissociation of contractile activity of the whole uterus from spike discharges. These results suggested to Kao (1976) that local groups of myometrial cells became electrically independent following progesterone treatment. This suggestion is supported by later observations that progesterone treatment caused a sharp reduction in the number of myometrial gap junctions (Garfield *et al.*, 1980). The progesterone-induced impairment in the spread of excitation explains the reduced amplitude and rate of intrauterine pressure cycles. Many of the effects of progesterone also may be related to its antagonism of estrogen actions, possibly at the level of estrogen receptors (Pavlik and Coulson, 1976; Clark *et al.*, 1977).

In part because of the ability of progesterone to maintain myometrial activity in a blocked state, Csapo proposed that the decline of circulating progesterone levels in species like the rat and rabbit results in increased excitability of the myometrium by lowering the threshold for excitation by endogenous oxytocic agents (Csapo, 1975). In the rat, ovariectomy (progesterone withdrawal) in late pregnancy resulted in premature labor, which was prevented by the administration of exogenous progesterone (Csapo and Wiest, 1969). The importance of progesterone withdrawal in the termination of gestation in rodents is further evident from observations that several pregnancy-interceptive agents operating by different mechanisms all reduce circulating progesterone concentrations (Creange *et al.*, 1978; Raziano *et al.*, 1972; Glasser *et al.*, 1972; Warnock and Csapo, 1975; Csapo and Resch, 1979; Fuchs *et al.*, 1974; Strauss *et al.*, 1975). The effects of these agents, which include inhibitors of progesterone synthesis, luteolytic prostaglandins, antisera to progesterone, and a progesterone antagonist, were reversed by the administration of progesterone. Only partial restoration of progesterone levels was required to block the ability of $PGF_{2\alpha}$ to induce premature labor in rats (Fuchs *et al.*, 1974; Alexandrova

and Soloff, 1980b). The reductic f plasma progesterone to as little as 20% the initial levels by $PGF_{2\alpha}$ treatment was compatible with undisturbed gestation, whereas a drop to 15% of the initial levels was associated with the termination of pregnancy (Fuchs *et al.*, 1974).

A fall in plasma progesterone concentrations near the end of gestation does not occur in all species (e.g., guinea pig and human). Progesterone also does not inhibit myometrial activity in the guinea pig (Zarrow *et al.*, 1963; Schofield, 1964). Csapo (1975) argued that measurement of blood levels of progesterone in species in which the steroid is produced by the placenta may not reflect the concentrations seen at uterine target cells because progesterone presumably is transported to the uterus locally. Infusion of progesterone into the maternal circulation may not increase the amounts available locally. Despite the lack of a preparturitional fall in blood progesterone levels in humans and the absence of any effect of progesterone administration on the length of gestation, an important role for progesterone is illustrated by the ability of a progesterone antagonist, RU486, to initate labor in humans. RU486, which inhibits progesterone action by competing for receptor sites, induced labor and delivery in women with dead fetuses at about 24 weeks of pregnancy (Cabrol *et al.*, 1985). Although RU486 also antagonizes the effects of glucocorticoids, the dose used minimized this effect. These findings suggest that progesterone might play an important role in labor in humans, as in other species. The actions of progesterone in humans may not be regulated by levels of the steroid in the blood but by changing levels of its receptors.

The production and metabolism of progesterone and estrogen by intrauterine tissues have been studied with the idea that they act on uterine cells in a paracrine fashion. Human amnion, chorion, and decidua converted pregnenolone to progesterone (Gibb *et al.*, 1978; Mitchell *et al.*, 1982). The conversion by either dispersed cells from chorion and decidua or explants maintained in culture was lower in tissues obtained at spontaneous labor than in tissues obtained at term but before labor (elective cesarean section) (Challis and Vaughan, 1987; Mitchell *et al.*, 1987). Because there was no increased rate of progesterone metabolism by human fetal membranes (Milewich *et al.*, 1977), the reduced rate of synthesis may bring about "local progesterone withdrawal." The reduced conversion of pregnenolone to progesterone by human fetal membranes *in vitro* during spontaneous labor may be the result of estrone or estradiol inhibition. Both estrogens are increased locally at the end of term (Mitchell *et al.*, 1982).

3.1.1. Mechanisms of Progesterone Withdrawal

In the cow, rabbit, goat, and rat, the major source of progesterone in peripheral plasma is the corpus luteum (for a review see Thorburn and Challis, 1979), and a mechanism exists for the reduction of luteal production of progesterone. In the goat and sheep, the fetus appears to control partutition through adrenal cortisol output (Thorburn and Challis, 1979). Cortisol-stimulated production of uterine prostaglandins may result in a luteolytic effect with a resultant decrease in progesterone concentrations.

In rats near term, there was an increase in ovarian 20α-hydroxysteroid dehydrogenase and in the corresponding conversion of progesterone to 20α-dihydroxyprogesterone (Wiest *et al.*, 1968). The drop in progesterone levels in the blood was accompanied by an increase in 20α-dihydroxyprogesterone, which has greatly reduced progestational potency (Wiest *et al.*, 1968). Prostaglandin F_{α}-induced luteolysis in pregnant rats appears to be mediated by an increase in ovarian 20α-hydroxysteroid dehydrogenase activity and a significant drop in plasma progesterone concentrations (Strauss and Stambaugh, 1974). There is strong evidence indicating that $PGF_{2\alpha}$ from the uterus is the physiological mediator of luteolysis in the rat. Prostaglandin $F_{2\alpha}$ is luteolytic in pseudopregnant rabbits, causing a decline in corpus luteum weight and progesterone secretion (Scott and Rennie, 1970; Gutknecht *et al.*, 1972). The administration of the prostaglandin synthesis inhibitor indomethacin prolonged the activity of the corpus luteum in pseudopregnant rabbits (O'Grady *et al.*, 1972). The decline in luteal progesterone secretion

in late pregnancy in the rabbit may be determined by withdrawal of luteotropic support by estrogens. In the rabbit, estrogens are luteotrophic (Hilliard, 1973); administration of the antiestrogen tamoxifen to pregnant rabbits produced a rapid fall in plasma progesterone and caused premature labor (Furr *et al.*, 1976). The sharp decline in progesterone output by the corpus luteum of the rabbit in late pregnancy may result from a combination of increased luteolytic and diminished luteotrophic activities.

Progesterone withdrawal is not limited to species that depend on the corpus luteum for its production. In sheep, progesterone production after day 50 of pregnancy is primarily by the placenta, but plasma progesterone concentrations declined in a variable fashion over the last 5–15 days of pregnancy, following the increase in fetal plasma cortisol (for reviews see Thorburn and Challis, 1979; Liggins *et al.*, 1973). Premature parturition induced by the administration of either ACTH or glucocorticoids to the fetus was preceded by a fall in maternal progesterone concentrations. In sheep, the fall in progesterone concentration is accompanied by a rise in the concentration of the progesterone metabolite 17α,20α-dihydroxyprogesterone.

Taylor *et al.* (1982) used an inhibitor of 3β-hydroxysteroid dehydrogenase activity to induce progesterone withdrawal in sheep during late pregnancy. They found that a decrease in plasma progesterone preceded premature delivery in most animals. The major site of action of progesterone in pregnant sheep has been suggested to be the maternal component (decidua) rather than the myometrium (Liggins *et al.*, 1973).

3.1.2. Changes in the Balance between Estrogen and Progesterone

Initiation of labor in sheep, goats, and cows is linked to maturation of the fetal hypothalamopituitary–adrenal axis, which results in an increase in fetal cortisol production (for reviews see Liggins *et al.*, 1973; Thorburn and Challis, 1979). Cortisol appears to induce 17-OH hydroxylase activity, resulting in suppression of either placental (sheep) or ovarian (goat) progesterone production and a concomitant stimulation in estrogen secretion. The net result is a hormonal environment favorable to the stimulation of uterine contractions and the termination of gestation, including increased $PGF_{2\alpha}$ production by uterine tissues. Elevations in plasma glucocorticoid, either by direct administration or by administration of ACTH to pregnant sheep, results in premature labor and hormone changes similar to those observed before spontaneous parturition. Glucocorticoids, however, do not appear to be directly involved in the initiation of labor in most other species studied. Maternal plasma cortisol levels essentially remain unchanged in primates during pregnancy, and glucocorticoids administered to monkeys do not induce premature labor (Novy, 1983). Treatment of women in the last trimester of pregnancy with a synthetic corticosteroid for prevention of idiopathic respiratory syndrome likewise did not influence the timing of the onset or the duration of labor (Gennser *et al.*, 1977). Murphy (1982) measured cortisol levels in umbilical cord serum arising from the fetus. She found a steady rise in fetal cortisol levels with increasing gestational age in late pregnancy, regardless of whether labor occurred. The sharp increase in the concentration of cortisol in monkeys on the day of vaginal delivery (Novy, 1983) has been attributed to maternal or fetal stress associated with parturition.

3.2. Relaxin

One of the effects of relaxin in a number of species, when administered either *in vivo* or *in vitro*, is the inhibition of spontaneous uterine contractions (for reviews see Schwabe *et al.*, 1978; Bryant-Greenwood 1982). Relaxin added to a muscle bath reduced the amplitude of contractions of rat uterine strips (Sawyer *et al.*, 1953). *In vivo*, relaxin decreased the frequency of contractions (Porter *et al.*, 1979). Extracts of human corpora lutea of pregnancy inhibited

the spontaneous activity of m[illegible]etrial strips from nonpregnant women ([illegible]chter *et al.*, 1980), suggesting that relaxin may also have some significance in the human.

Relaxin may be biologically important in preventing premature uterine contractions. Relaxin administered to estrogen-treated ovariectomized rats abolished myometrial activity that was induced by infusions of either oxytocin or $PGF_{2\alpha}$ (Porter *et al.*, 1981). Apparently this phenomenon can be observed only *in vivo*, because the addition of oxytocin or prostaglandins to rat myometrial strips overcame the inhbitory effects of relaxin *in vitro* (Chamley *et al.*, 1977). The *in vivo* and *in vitro* differences of the overriding effects of uterotonic agents on the inhibitory activity of relaxin may be more apparent than real and result from differences in the concentrations of uterotonic agents that are effective *in vivo* and *in vitro*. The effect of relaxin is highly dependent on the uterine response to the dose of oxytocin or $PGF_{2\alpha}$. Sanborn (1986) pointed out that concentrations of oxytocin and $PGF_{2\alpha}$ that promoted only phasic contractions *in vitro* were antagonized by relaxin, whereas relaxin was ineffective as an antagonist at higher concentrations of these uterotonic agents, which caused stronger, tonic uterine contractions.

Relaxin may be important in keeping the myometrium quiescent. The quiescence seen in several species shortly before parturition corresponds temporally with high levels of relaxin in the circulation (Downing and Sherwood, 1985b). Downing and Sherwood (1985b) showed that increasingly prolonged periods of myometrial quiescence that occur in the intact rat during the course of pregnancy, until about 3 hr prepartum, could be mimicked by the administration of porcine relaxin, estradiol, and progesterone to ovariectomized pregnant rats. Without relaxin, there was a significant increase in myometrial activity from day 12 through the remainder of pregnancy. The animals treated with estradiol, progesterone, and relaxin exhibited a pattern of myometrial activity during labor that was similar to that of intact controls.

Relaxin may also contribute to myometrial quiescence by inhibiting oxytocin release. In anesthetized lactating rats, intravenous injections of porcine relaxin suppressed the onset of reflex milk ejection, which is dependent on circulating oxytocin concentrations (Summerlee *et al.*, 1984). Mammary sensitivity to exogenous oxytocin was reduced by relaxin treatment, but not sufficiently to explain the effects observed after relaxin administration. Because injection of relaxin into the cerebral ventricles disturbed the pattern of reflex milk ejection without affecting the response of the mammary gland to oxytocin, the authors suggested that relaxin has a central action on the release of oxytocin. Additional studies showed that inhibition of oxytocin release also occurred after chronic infusion of porcine relaxin into conscious lactating rats (Jones and Summerlee, 1987).

The effects of relaxin on uterine smooth muscle are apparent only after estrogen priming (for a review see Schwabe *et al.*, 1978). Estrogens up-regulate the concentration of relaxin receptors in the myometrium (Mercado-Simmen *et al.*, 1980). The concentration of relaxin receptors in rat myometrial plasma membrane fractions was high at proestrus and estrus and nondectectable for the rest of the cycle (Mercado-Simmen *et al.*, 1980). On the basis of these findings, relaxin receptor levels should be suppressed throughout most of pregnancy, during progesterone domination, and rise sharply near term with the sudden onset of estrogen domination. Instead, receptor levels began to rise at about 15 days, peaked at 17 days, and declined thereafter (Mercado-Simmen *et al.*, 1980). These findings indicate that other factors also may be involved in regulation of relaxin receptor concentrations. Relaxin may down-regulate its own receptor (Mercado-Simmen *et al.*, 1980). The relationship between uterine quiescence and relaxin receptor concentration is uncertain because uterine quiescence is greatest at times when relaxin receptor concentrations are declining.

Relaxin, administered either *in vitro* or *in vivo*, produced small but significant increases in rat uterine cAMP levels (Sanborn *et al.*, 1980; Cheah and Sherwood, 1980; Judson *et al.*, 1980). The increases in intracellular cAMP concentrations are postulated to result in myometrial cell relaxation, by analogy with the mechanism of isoproterenol-induced relaxation.

As with other smooth muscle cells, elevation of intracellular Ca^{2+} concentrations initiates contractions of the myometrial cell by the following mechanism. Increased Ca^{2+} binding

tc modulin results in a $Ca^{2+}{}_4$-calmodulin co ex that binds to and activates myosin light-chain kinase, which catalyzes the phosphorylation of the 20-kDa light-chain subunit of myosin. Myosin phosphorylation results in stimulation of actin-activated ATPase and actin–myosin interactions.

Adelstein and colleagues (1978) observed that avian smooth muscle myosin light chain kinase was phosphorylated by cAMP-dependent protein kinase, resulting in a tenfold increase in the concentration of Ca^{2+}–calmodulin required for half-maximal activation of myosin light chain kinase. Myosin light chain kinase phosphorylated by cAMP-dependent protein kinase requires higher concentrations of cytoplasmic Ca^{2+} for activation. This decrease in sensitivity to Ca^{2+} can result in relaxation or inhibition of contraction and may explain why elevation of intracellular levels of cAMP relaxes uterine smooth muscle. There are, however, observations that are not consistent with the regulation of smooth muscle contractility by cAMP levels: (1) the rate of dissociation of Ca^{2+}–calmodulin from myosin light chain kinase is much slower than the relaxation produced by agents that increase cAMP (Kamm and Stull, 1985); (2) isoproterenol-induced increases in cAMP levels in myometrial cells in culture occurred more than five times faster than relaxin-induced increases (Hsu *et al.*, 1985); (3) cAMP levels were unchanged under conditions of inhibition of contractile activity by relaxin. These findings suggest either that very small changes in cAMP concentrations are effective in mediating relaxin action or that cAMP is not a mediator. The role of cAMP in uterine relaxation is further complicated by the observation that prostaglandins, which stimulate uterine contractions, also caused an elevation in myometrial cell cAMP levels (Vesin *et al.*, 1978). Nishikori *et al.* (1983) showed that relaxin added to the medium bathing myometrial strips from estrogen-primed, $PGF_{2\alpha}$-treated rats decreased the ratio of phosphorylated to nonphosphorylated 20-kDa myosin light chain. Relaxin also decreased myosin light chain kinase activity. The decreases in both the relative amount of phosphorylated light chain and kinase activity paralleled relaxin inhibition of contractile activity with respect to both time and dose.

3.3. *Adrenergic Agents*

The myometrial response to sympathetic stimulation shifts from contraction in the nonpregnant state to relaxation late in pregnancy. It is now clear that these effects occur through selective activation of α- and β-adrenergic activities, respectively (Ahlquist, 1966). The shift during pregnancy appears to be the result of changing levels of estrogen and progesterone. Estrogen administration enhanced the contractile response of immature rabbit uterus to hypogastric nerve stimulation *in vitro*, whereas progesterone given to estrogen-primed rabbits inhibited spontaneous uterine contractions (Miller and Marshall, 1965). The shift from α to β responses appeared to result in part from modifications of adrenergic receptor concentrations. In the rabbit, the α-adrenergic receptor concentration increased threefold after administration of estrogen, and the estrogen effect was inhibited by progesterone (Roberts *et al.*, 1981; Williams and Lefkowitz, 1977). Estrogen administration had no effect on the number of β-adrenergic receptors. The importance of α-adrenergic receptor regulation in determining an α-adrenergic response, however, is not clear. In the rabbit the increase in α-adrenergic receptor caused by estrogen administration consisted only of an increase in the α_2 subtype, whereas uterine contractions appear to be mediated by the α_1 subtype (Hoffman *et al.*, 1981). In the rat, estrogen administration caused an increase only in the number of myometrial β-adrenergic receptors (Krall *et al.*, 1978). Thus, the mechanisms of steroid regulation of the response to α-adrenergic agents still require clarification.

It also does not appear that β-adrenergic dominance under the influence of progesterone can be explained by receptor changes. Neither the absolute concentration of β receptors nor the ratio of α to β receptors appears to account for the predominance of a β-adrenergic response (Roberts *et al.*, 1981). Progesterone also did not appear to affect the coupling between β

receptors and guanosyl nucl de regulatory proteins because the affinity he receptors for isoproterenol was not affected by progesterone treatment.

The role of adrenergic stimulation of the uterus during pregnancy is not known. Pregnancy lowers the norepinephrine content, tyrosine hydroxylase activity, axonal uptake of [^{3}H]norepinephrine, and the number of adrenergic nerves in the myometrium (Thorbert, 1978). Complete nerve degeneration occurs by the end of gestation (Thorbert *et al.*, 1979). Progesterone, when elevated for extended periods as in pregnancy, also induces a functional sympathetic denervation of the myometrium (Bell and Malcolm, 1978). Consequently, neural activity has no influence on myometrial function at the time of parturition.

β_2-Adrenergic agonists are among the more useful agents in the treatment of premature labor in humans (see Falck Larsen *et al.*, 1986). The β-mimetic drug ritodrine inhibited preterm labor in the initial stage, resulting in a gain of a few days to a few weeks in length of gestation (Falck Larsen *et al.*, 1986). The ability of β-mimetic therapy to prolong gestation significantly in preterm labor, however, is equivocal (Spellacy *et al.*, 1979; Falck Larsen *et al.*, 1980). Myometrial relaxation followed by desensitization with return of myometrial contractions results from continuous exposure to β-adrenergic agonists both *in vivo* and *in vitro* (Andersson *et al.*, 1980). Selective β_2 agonists inhibit myometrial contractibility by activation of receptor-mediated adenylate cyclase, leading to increased cAMP content (Andersson *et al.*, 1980; Harden 1983). The mechanism of desensitization is not completely understood. Down-regulation of β receptors, decrease in adenylate cyclase activity, and modifications in interactions of receptor or adenylate cyclase with G proteins may be involved (Harden, 1983).

4. *Activators of Uterine Activity*

4.1. *Prostaglandins*

Prostaglandins are produced by nearly all mammalian cells except erythrocytes. Prostaglandins are not stored but are released immediately after synthesis. They act locally and are metabolized by the same cells that produce them or by neighboring cells, and they enter the systemic circulation only as inactive products. Those primary prostaglandins that escape local metabolism are broken down to inactive metabolites after a single pass through the lungs, which have high concentrations of 15-hydroxyprostaglandin dehydrogenase and 13,14-dehydroprostaglandin reductase.

Prostaglandins play an important role in parturition. Following the discovery of prostaglandins in amniotic fluid and in the circulation of women in labor (Karim and Devlin, 1967), Karim proposed that $PGF_{2\alpha}$ was involved in spontaneous labor (Karim, 1971). Prostaglandin$_{2\alpha}$ is used for labor induction and for the induction of abortion between the ninth and 22nd weeks of pregnancy (for references see Karim, 1971).

Because treatment of women with PGE_2 or $PGF_{2\alpha}$ stimulates uterine contractions at any stage of gestation, many investigators assumed that endogenous prostaglandins were involved in the initiation of labor. Indeed, gestation could be extended in a number of species by drugs that block prostaglandin synthesis (Aiken, 1972; Chester *et al.*, 1972; Lewis and Schulman, 1983; Waltman *et al.*, 1973; Novy *et al.*, 1974; Zuckerman *et al.*, 1974; Wiqvist, *et al.*, 1975). Stimuli known to cause prostaglandin release, such as cervical manipulation, stripping of the chorion laeve from the contiguous decidua, and infections of the membranes, usually result in labor near term (Mitchell *et al.*, 1977).

Substantial increases in the concentration of prostaglandins or their metabolites in amniotic fluid (Keirse and Turnbull, 1973; Keirse *et al.*, 1977) and in the maternal circulation (Gréen *et al.*, 1974; Lackritz *et al.*, 1978) during advanced labor have suggested a causal

relatic ip between prostaglandin release and uter. activity. However, *in vivo* production and concentrations of prostaglandins in the intrauterine environment have been difficult to evaluate because of the presence of prostaglandin-synthesizing and -metabolizing enzymes. For example, cellular trauma caused by handling tissues is a major stimulus of prostaglandin production (Piper and Vane, 1971). Abnormally high rates of prostaglandin release may, therefore, be observed for some time after removal of a tissue. The effect of handling was minimized by use of cells separated from tissues and maintained in culture, cultured organ explants, or perifused tissues. Prostaglandin production usually stabilizes at a lower level after an initial burst. Unfortunately, modification of the natural milieu and the interjection of a time interval between obtaining the tissues and making the measurements may alter the characteristics of the response.

Prostaglandin E_2 and $PGF_{2\alpha}$ are produced in largest amounts by uterine and intrauterine tissues during human parturition. The measurement of these prostaglandins in peripheral plasma also is hampered by their low concentrations, rapid clearance from the bloodstream, and the contribution of platelets to prostaglandin production. Estimates of $PGF_{2\alpha}$ levels have been obtained more reliably by measurement of the stable metabolite, PGFM (13,14-dihyro-15 keto $PGF_{2\alpha}$) (Levine and Gutierrez-Cernosek, 1973; Cornette *et al.*, 1974; Samuelsson *et al.*, 1975). The estimate of PGE_2 in peripheral maternal plasma has become more reliable with the development of assays for the stable metabolite, bicyclo-PGEM (11-deoxy-13,14-dehydro-15-keto-13-11β-16ξ-cyclo-PGE_2) (Fitzpatrick *et al.*, 1980; Granström *et al.*, 1980; Bothwell *et al.*, 1982).

Prostaglandin-synthesizing and -degrading enzyme activities are absent in amniotic fluid (Keirse and Turnbull, 1975). Measurement of prostaglandin levels in amniotic fluid, therefore, has been one of the most popular approaches to ascertaining changes in the initiation and progression of labor. Contributing to prostaglandin levels in amniotic fluid are the amnion, which produces primarily PGE_2 (Mitchell *et al.*, 1978; Okazaki *et al.*, 1981; Casey *et al.*, 1984), prostaglandins and metabolites from fetal urine, presumably arising from fetal kidneys (Casey *et al.*, 1983a), and prostaglandins and metabolites produced by other uterine and intrauterine tissues.

Investigators have also measured the rates of production of a variety of prostanoids by intrauterine tissues *in vitro*, both before and after establishment of labor. The rate of production of PGE_2 by amnion homogenates or cultured cells was greater when taken at or after labor than in late gestation in the absence of labor (Okazaki *et al.*, 1981a; Olson *et al.*, 1983a). Likewise, the concentration of prostaglandins in the first voided urine of human babies was greater in infants born after spontaneous labor than in those delivered by cesarean section in the absence of labor (Casey *et al.*, 1983a). These findings were consistent with the rise in amniotic fluid PGE_2 and $PGF_{2\alpha}$ levels during term labor (Keirse and Turnbull, 1973; Keirse *et al.*, 1977).

In favor of a labor-initiating role for prostaglandins are the findings that there was a sharp prelabor rise in $PGF_{2\alpha}$ and PGFM in ewe uterine venous blood (Liggins *et al.*, 1973) and peripheral circulation (Mitchell *et al.*, 1976a), respectively. In rhesus monkey amniotic fluid, levels of PGF and its metabolite began to rise a few days before parturition (Mitchell *et al.*, 1976b). It is not clear, however, whether prostaglandin levels increase in humans before labor. Although some investigators have suggested that $PGF_{2\alpha}$ levels in human amniotic fluid rose before the onset of labor (Keirse *et al.* 1977), the results of most studies have shown that there was a marked rise in plasma PGFM levels only during or after labor (Gréen *et al.*, 1974; Lackritz *et al.*, 1978; Fuchs *et al.*, 1983b; Sellers *et al.*, 1982). Fuchs *et al.* (1983b) reported that plasma PGFM levels did not increase in women until cervical dilatation was about 6 cm, both in patients with intact membranes and in those with ruptured membranes. These observations suggest that prostaglandins may be released only as a consequence of labor in humans.

Other observations suggest that endogenous prostaglandins are not associated with labor initiation in humans. Many women with premature rupture of the membranes and increased

prostaglandin metabolite levels their circulation did not go into labor spineously but required oxytocin stimulation (Husslein *et al.*, 1981). The same was true in some women after artificial rupture of membranes (Husslein *et al.*, 1983). Padayachi *et al.* (1986) showed that oxytocin administration to patients with comparable levels of cervical dilation and with delayed labor initiated uterine contractions and permitted normal vaginal delivery without materially affecting PGF or PGFM levels in amniotic fluid. Only after efficient uterine contractions had continued for several hours was there an increase in prostaglandins in amniotic fluid. On the other hand, Husslein *et al.* (1981) and Fuchs *et al.* (1983b) found that induction of labor by oxytocin was associated with a rise in circulating levels of PGFM. When there was no increase in PGFM levels, induction of labor with oxytocin was unsuccessful. Taken together, the findings suggest that $PGF_{2\alpha}$ does not play a role in initiating labor but may be related to events associated with labor. Release of $PGF_{2\alpha}$ from extraamniotic sites, probably the decidua, may be more relevant with regard to these events than prostaglandins produced by the amnion. In the human, endogenous prostaglandins may support the initiation of parturition by oxytocin and other factors or maintain labor once it has begun.

4.1.1. *Sites of Prostaglandin Action*

Because prostaglandins are uterotonic, endogenously released prostanoids have been assumed to act directly on uterine myometrial cells to elicit labor contractions. Their actions on myometrial cells, however, may not be primary. Intravenous infusion of prostaglandins into women stimulated uterine activity only after a latency period of 15–20 min (Embrey, 1969), and the uterotonic effect persisted for 30–60 min after the infusion was stopped. In addition, blood levels of prostaglandins attained during infusion for the induction of labor are too low to promote uterine contractions directly.

4.1.2. *Induced Labor in Humans*

Nagata and co-workers (1987) compared PGFM levels in the circulation of women with induced and spontaneous labor. Although prostaglandin levels increased only during or after delivery in spontaneous labor, there was a significant elevation when labor was induced by amniotomy. These findings suggest that $PGF_{2\alpha}$ may play different roles in induced and spontaneous labor. In bacterial sepsis, Bejar *et al.* (1981) showed that many bacteria associated with intrauterine infections, chorioamnionitis, urinary tract infections, and early neonatal sepsis have substantial phospholipase activities, some of which were several times greater than phospholipase A_2 activities in amnion and chorion. These workers postulated that premature labor associated with endocervical or intrauterine infections could arise from bacterially induced release of prostaglandins.

4.1.3. *Sites of Prostaglandin Synthesis in Labor*

4.1.3a. Myometrium. Because virtually all cells are capable of prostaglandin synthesis, the question of the sources of prostaglandins involved in myometrial contractions has not been completely resolved. If prostaglandins are to affect myometrial contractility, they should arise from or very near to myometrial cells. There is little evidence to suggest that the myometrium itself is the source of prostaglandins that are involved in labor. Whereas the endometrium and decidua synthesize predominantly $PGF_{2\alpha}$ and PGE_2 in almost equal amounts (Okazaki *et al.*, 1981), the myometrium produces primarily prostacyclin (PGI_2) (Williams *et al.*, 1978; Abel and Kelly, 1979; Omini *et al.*, 1979). Prostacyclin administered either *in vivo* (Lumsden and Baird, 1986) or *in vitro* (Omini *et al.*, 1979) relaxed uterine smooth muscle or induced an initial excitatory response followed in the majority of experiments by transient

inhi... .on (Wikland *et al.*, 1983). The inhibitory ...fects of PGI_2 have not been universally observed, however. Using strips of pregnant rat uterus, Williams *et al.* (1979) found that PGI_2 stimulated uterine contractions, and threshold concentrations caused a threefold potentiation of threshold doses of oxytocin. Protacyclin may not be a physiological activator of uterine contractions because the uterotonic potency of PGI_2 is about one-eighth that of $PGF_{2\alpha}$ and 1/30 that of PGE_2. Others have found that PGI_2 had no effect on human uterine contractility *in vivo* (Wilhelmsson *et al.*, 1981).

Specific binding sites for $[^3H]PGI_2$ have been demonstrated by autoradiography in the myometrium but not the endometrium of nonpregnant human uteri (Chegini and Rao, 1988). Prostaglandin E_2, $PGF_{2\alpha}$ and leukotriene C_4, which bind to nonpregnant human uterus (Hofmann *et al.*, 1983), had no effect on PGI_2 binding, suggesting that there are separate receptor sites for the different eicosanoids (Chegini and Rao, 1988). The biological significance of prostacyclins and their binding sites in the myometrium remains to be established. Prostacyclin formation in human myometrium may modulate the stimulatory activities of prostaglandins and other hormones during pregnancy (Omini *et al.*, 1979). Prostacyclin abolished spontaneous electrical and mechanical activity *in vivo* in sheep within minutes of administration; however, it did not block the response of the myometrium to oxytocin or $PGF_{2\alpha}$ (Lye and Challis, 1982). Prostacyclin also did not block the contractile activities of $PGF_{2\alpha}$ and oxytocin on human myometrial strips (Wikland *et al.*, 1983).

The fetal membranes, notably the amnion, or decidua may be the source of prostaglandins involved in labor. Implicit in the argument for extramyometrial production of activator prostaglandins is the existence of some mechanism for transport of prostaglandins to the myometrium without inactivation.

4.1.3b. Amnion. Amniotic fluid is surrounded by tissues that do not metabolize prostaglandins (Keirse and Turnbull, 1973). Keirse *et al.* (1977) showed that in women concentrations of PGF and PGFM in amniotic fluid were higher at the onset of labor than in late pregnancy and increased significantly with advancing cervical dilatation. Similar findings were made in rhesus monkeys. The mean concentrations of PGF and PGFM in amniotic fluid of rhesus monkeys increased about fourfold during the last 5 days of pregnancy (Mitchell *et al.*, 1976b).

Some investigators have suggested that the amnion is the major site of increased prostaglandin production at the time of labor (Casey and MacDonald, 1984). These conclusions were based in part on the large capacity for prostaglandin synthesis by amnion cells (Kinoshita *et al.*, 1977; Willman and Collins, 1978; Mitchell *et al.*, 1978; Okazaki *et al.*, 1981), and the production rates of PGE by dispersed amnion cells were significantly higher after spontaneous labor at term than at elective cesarean section (Okazaki *et al.*, 1981; Olson *et al.*, 1983a; Manzai and Liggins, 1984). Other studies, however, have shown no statistically significant difference in amniotic PGE production rates before and after labor at term (Mitchell *et al.*, 1978). Reddi *et al.* (1984) showed that multiparous patients with comparable levels of cervical dilatation and with delayed labor had very low concentrations of $PGF_{2\alpha}$ in the amniotic fluid, suggesting that the delay might be associated with low levels of prostaglandin. Oxytocin administration restored uterine contractions to normal and permitted normal vaginal delivery but did not materially affect the low level of PGF and its metabolite PGFM in amniotic fluid (Reddi *et al.*, 1984; Padayachi *et al.*, 1986). Only after efficient uterine contractions had occurred for several hours was there an increase in prostaglandins. Results such as these question whether prostaglandin levels in amniotic fluid reflect the events leading up to the initiation of labor contractions.

4.1.3c. Decidua. Peripheral blood levels of PGFM increased slightly in late human pregnancy and greatly during labor or immediately post-partum (Greén *et al.*, 1974). It is not certain which cells were the source of PGF, but it is likely that they arose from the decidua.

Collagenase-dispersed decidual cells exhibited higher rates of PGE_2, $PGF_{2\alpha}$ and PGFM production when the tissue was taken from patients in spontaneous labor than at the time of elective cesarean section when the patients were not in labor (Skinner and Challis, 1985).

4.1.3d. Transport of Prostaglandins from Fetal Membranes to Myometrium. The increase in fetal membrane production of prostaglandins has led to speculation that the availability of prostanoids to the myometrium increases about the time of the onset of labor. Intraamniotic injection of prostaglandins is known to induce labor in a number of circumstances (Karim, 1971), presumably by simulating the physiological conditions associated with spontaneous labor.

In decidua, PGE_2 originating from the amnion may be converted to $PGF_{2\alpha}$ through 9-ketoreductase activity or may stimulate further arachidonate metabolism. Prostaglandin E_2 or $PGF_{2\alpha}$ from decidua may then reach the myometrium. An objection to the importance of amniotic PGE_2 acting on the myometrium is that human chorion contains high 15-prostaglandin dehydrogenase and $\Delta^{13,14}$-reductase activities and exhibits a high rate of prostaglandin metabolism (Keirse and Turnbull, 1975; Okazaki *et al.*, 1981). Nakla *et al.* (1986), however, showed that [^{3}H]PGE_2 was able to traverse full-thickness human fetal membranes (amnion, chorion, and decidua) partitioning two chambers *in vitro*. After the introduction of [^{3}H]PGE_2 on the amnion side, about half of the radioactivity recovered on the decidual side was identified as [^{3}H]PGE_2. The rate of transport was significantly higher in tissues collected at spontaneous onset of labor than in tissues taken at elective cesarean section at term. These authors conclude that PGE_2 produced by human amnion at term may escape metabolism in the chorion and reach the decidua, myometrium, or both.

In contrast, McCoshen *et al.* (1987), using a dual-compartment perfusion chamber, found that the release of endogenous PGE_2 on the decidual side of fetal membranes diminished after spontaneous labor despite an increased release from the fetal (amnion) surface. In support of these *in vitro* findings, they found that the PGE_2 content of chorion–decidua taken at the time of vaginal deliveries was one-quarter that from tissues taken during non-labor-associated cesarean deliveries. From these results, there is little likelihood that PGE_2 arising from amnion cells plays a role in initiating uterine contractions, emphasizing the importance of decidual prostaglandins as the source of myometrial activating agents.

4.1.3e. Amnion versus Decidua. The site of origin of prostaglandins involved in labor is still being resolved. Because little or no $PGF_{2\alpha}$ is produced by fetal membranes, and levels of PGFM increase in the amniotic fluid and maternal circulation around the time of parturition, many investigators have concluded that the decidua is the major site of prostaglandin production involved in parturition. In support of this view, Brennecke *et al.* (1985) found that levels of the stable metabolite of PGE_2 were not significantly altered in human maternal peripheral plasma during the second or third trimesters or labor. These findings contrasted with levels of PGFM, which were increased severalfold during labor. The presence of $PGF_{2\alpha}$ and PGFM in amniotic fluid during labor appears to result from its influx from extraamniotic sources because a significant fraction of PGE_2 applied to the vagina reached the amniotic fluid compartment unchanged after a lag period of a few hours (MacKenzie and Mitchell, 1981). It seems unlikely that $PGF_{2\alpha}$ in decidual tissue could be synthesized from PGE_2 arising from the amnion, because the degradation of PGE_2 by 15-ketoprostaglandin dehydrogenase appears to be favored over the conversion to $PGF_{2\alpha}$ by 15-ketoprostaglandin dehydrogenase (Niesert *et al.*, 1986).

Whether prostaglandins in amniotic fluid play any part in the mechanisms of the onset and progression of labor is unknown. The concentrations of PGE (Keirse and Turnbull, 1973) and PGF and PGFM (Keirse *et al.*, 1977) increased in amniotic fluid in proportion to cervical dilatation. Although the increases in PGF and PGFM began before the apparent onset of spontaneous labor, the increase in prostaglandin concentrations in amniotic fluid reflects the

progression of labor and probably not the initiation. Indeed, prostaglandins can be released as a result of intrauterine pressure and stretching or manipulation of the uterus (Poyser *et al.*, 1971). In agreement with these results, cervical dilatation at either weeks 9 to 12 or 37 to 42 of pregnancy resulted in a rapid, 13-fold elevation in PGF, but not PGE, concentrations in human amniotic fluid (Nieder and Augustin, 1983). Regardless of the biological significance of prostaglandins in amniotic fluid, their concentrations are an index of important changes during labor, since abnormally low levels of prostaglandins are found in the amniotic fluid of women with clinically delayed labor (Keirse *et al.*, 1977; Reddi *et al.*, 1984). There is no strong evidence, however, to suggest that the participation of the decidua or amnion in labor need exclude the other.

4.1.4. Uterotonic Effects of Prostaglandins and Myometrial Prostaglandin Receptors

Because prostaglandins stimulate myometrial contractions, increased levels of prostaglandins arriving at the myometrium were assumed to stimulate labor. There are observations, however, that suggest that the effects of endogenously released prostaglandins on the myometrium are indirect. Prostaglandin-induced contractions of the isolated uterus of the rat are characterized by a rapid onset and rapid recovery after washing (Johnson *et al.*, 1974). *In vivo*, however, there is a lag of about 48 hr between the administration of $PGF_{2\alpha}$ and induction of labor (Alexandrova and Soloff, 1980b). Prostaglandin $F_{2\alpha}$ does not induce labor when given to rats after day 21 of gestation, a time when oxytocin is very effective (Fuchs, 1972). In addition, PGE_2 and $PGF_{2\alpha}$ are almost equipotent uterotonic agents in the rat, but only $PGF_{2\alpha}$ terminated gestation when administered on day 18 (Fuchs *et al.*, 1974).

The effects of $PGF_{2\alpha}$ on the myometrium in the rat appear to result, at least in part, from its luteolytic activity (Pharriss and Wyngarden, 1969; Fuchs *et al.*, 1974; Strauss *et al.*, 1975). The simultaneous administration of progestin and $PGF_{2\alpha}$ prevented $PGF_{2\alpha}$-induced premature labor (Strauss *et al.*, 1975; Alexandrova and Soloff 1980b). Although evidence suggests that $PGF_{2\alpha}$ inhibits progesterone secretion in nonprimates (Horton and Poyser, 1976), similar effects could not be demonstrated in humans between 7 and 20 weeks of pregnancy (Speroff *et al.*, 1972).

The action of prostaglandin is mediated by specific receptors located on the plasma membranes of target cells. Specific binding sites for PGEs with apparent K_d values in the nanomolar range have been demonstrated in crude myometrial membrane preparations (Schillinger and Prior, 1976; Crankshaw *et al.*, 1979; Bauknecht *et al.*, 1981). Uterine binding sites were found autoradiographically in longitudinal and circular smooth muscle, stromal cells, glandular epithelium, arterioles, and erythrocytes within the lumen of the arterioles (Chegini *et al.*, 1986; Chegini and Rao, 1988). Prostaglandin $F_{2\alpha}$ binding by human myometrial plasma membranes, however, was associated with relatively low-affinity sites (K_d in the micromolar range), suggesting that there are separate receptors for PGE and PGF (Schillinger and Prior, 1976). Although PGE_2 is several times as potent as $PGF_{2\alpha}$ in stimulating contractions of human myometrium (Embrey, 1969; Schillinger and Prior, 1976), the affinity of binding sites for PGE_2 was over 1000 times that for $PGF_{2\alpha}$. Prostaglandin $F_{2\alpha}$ may undergo modification before binding, but the lack of correlation between uterotonic potency and binding creates doubt about the physiological significance of the binding sites. Other investigators, however, have found that the affinity of myometrial membranes from pregnant women for $PGF_{2\alpha}$ was about 0.3 nM (Fukai *et al.*, 1984). Unlike myometrial receptors for other uterotonic agents, the number of PGE_2 binding sites was reduced to one-third after nonpregnant patients were treated with estradiol. The reduced binding capacity may result from occupation of the sites by endogenous PGEs, the production of which is stimulated by estrogen (Abel and Baird, 1980).

The relationship between the affinities of a series of prostaglandin analogues and their relative potencies as uterotonic agonists remains to be established. The mechanisms of pros-

taglandin–receptor interaction . subsequent steps are poorly understood. Regulation of prostaglandin action at the receptor level during pregnancy and parturition has not been investigated. Fukai *et al.* (1984) found that the concentration of high-affinity binding sites (K_d about 0.3 nM) for [^{3}H]PGF$_{2\alpha}$ on human myometrial membranes remained unchanged throughout pregnancy and labor. The myometrial PGF$_{2\alpha}$ binding capacity was about 3 to 5% that of oxytocin, which increased substantially as gestation advanced (Fukai *et al.*, 1984).

4.1.5. *Control of Prostaglandin Synthesis*

The control of prostaglandin release during labor remains unknown. Changes in prostaglandin output are not related to a decrease in prostaglandin metabolism. The capacity to produce prostaglandins from endogenous substrates exists long before labor begins. The increased production of prostaglandins may result from a change in the balance of stimulatory to inhibitory factors modulating prostaglandin biosynthesis.

4.1.5a. Stimulatory Factors. Stimulatory factors have been found in fetal urine (Strickland *et al.*, 1983; Casey *et al.*, 1983b), amniotic fluid (Rehnström *et al.*, 1983; Mitchell *et al.*, 1984), and the cytosol fractions of placenta and decidua (Saeed and Mitchell, 1982).

Casey and co-workers (1983b) found a protein or protein-associated material in human fetal urine that caused a ten- to 600-fold increase in PGE$_2$ synthesis by human amnion cells maintained in monolayer culture. Fetal urine did not cause a similar increase in PGE$_2$ production by cells derived from human myometrium or endometrium. The activity was also present in adult urine. Strickland *et al.* (1983) found a material in neonatal urine that stimulated the *in vitro* conversion of arachidonic acid to PGE$_2$ by a microsome-enriched preparation from bovine seminal vesicles. Stimulation was greater in urine of babies delivered after labor than by cesarean section before the onset of labor. These workers postulated that the resulting stimulation of prostaglandin synthesis by fetal membranes could serve as the signal for the initiation of parturition.

Rehnström et al. (1983) examined the influence of amniotic fluid on the synthesis of prostanoids by tissue slices of human amnion, decidua, and myometrium. They found that amniotic fluid taken at term, either before or after the onset of labor, stimulated prostaglandin synthesis by decidua and myometrium but not by amnion. Amniotic fluid taken at midtrimester was without activity. The stimulating effect could not be explained by free arachidonic acid in amniotic fluid. Arachidonate, which is known to increase in late pregnancy, would have stimulated prostaglandin synthesis by amnion. Other investigators (López Bernal *et al.*, 1987) showed that the addition of arachidonic acid to dispersed amnion cells resulted in a two- to threefold increase in PGE output, whether the cells were obtained at term either before or after labor. The absence of a significant stimulatory effect of amniotic fluid on amnion (Rehnström *et al.*, 1983) suggests that factors arising in amniotic fluid, presumably from the fetus, must reach the decidua and myometrium to be effective.

Arachidonic acid. Regulation of prostaglandin synthesis may be by arachidonic acid availability and the activities of enzymes involved in the conversion of arachidonic acid to prostaglandins (prostaglandin synthetase). Prostaglandins are formed from polyunsaturated fatty acids released from phospholipids in the plasma membranes of cells. An early step in the formation of prostaglandins is the formation of arachidonic acid from its esterified form in phospholipids, primarily phosphatidylethanolamine and phosphatidylinositol, through the activities of phospholipases A$_2$ and C, respectively. The activities of these enzymes increase during pregnancy, although not in association with labor *per se* (Okazaki *et al.*, 1981). Phospholipase A$_2$ is specific for phosphatidylethanolamine containing arachidonic acid in the *sn*-2 position. From arachidonic acid, a cyclopentane ring is formed, and oxygen atoms are introduced by prostaglandin synthetase to yield PGE$_2$ and PGF$_{2\alpha}$, the two prostaglandins of importance in parturition.

A rate-limiting step in the synthesis of prostaglandins is phospholipase-mediated cleavage of arachidonic acid from phospholipid stores, because the pool of free arachidonic acid in most mammalian cells is very small (Irvine, 1982). The binding of agonists to cell surface receptors is associated with phospholipase activation and increased production of prostaglandins (Samuelsson *et al.*, 1978; Hong and Deykin, 1981; Majerus, 1983). At least part of the increase in the production of prostaglandins after stimulation, however, results from increased prostaglandin synthetase activity, measured by the conversion of exogenous arachidonic acid to prostaglandins (see Habernicht *et al.*, 1985, for additional references).

It is not clear whether arachidonate is the limiting, and thus regulated, step of prostaglandin synthesis in fetal membranes and uterine tissues in parturition. In support of a rate-limiting role is the eightfold increase in concentration of nonesterified arachidonic acid in amniotic fluid during labor (MacDonald *et al.*, 1974). Because arachidonic acid increased much more than other fatty acids, the mobilization of arachidonic acid may be associated with labor. Although the specific activity of phospholipase C in fetal membranes and decidua did not change with the onset of labor in humans (Di Renzo *et al.*, 1981), the tacit assumption is that an increase in arachidonate levels results in synthesis of more prostaglandins. In support of this argument, the instillation of arachidonic acid into the amniotic sac resulted in abortion in midpregnancy (MacDonald *et al.*, 1974), whereas oleate instillation had no effect. Arachidonic acid increased PGE output significantly by amnion cells, whether the amnion was obtained from patients in spontaneous term labor, spontaneous preterm labor, induced labor, or at term but not in labor (Lopez Bernal *et al.*, 1987). These findings support the hypothesis that substrate availability is an important determinant of the rate of PGE synthesis.

Others, however, have shown that human amniotic fluid and uterine tissues contain a great excess of arachidonic acid compared with PGE and PGF. Arachidonic acid in both esterified and free forms accounted for between 7% and 25% of the fatty acid content (Keirse, 1983). Extraamniotic instillation of arachidonic acid into pregnant rhesus monkeys was ineffective in elevating prostaglandin concentrations in either amniotic fluid or peripheral plasma or in inducing labor prematurely (Robinson *et al.*, 1978), suggesting that the formation of arachidonate is not limiting. In contrast, the extraamniotic administration of PGE_2 resulted in increased concentrations of PGE, PGF, and PGFM in amniotic fluid, and labor was induced prematurely. The basis for the discrepancy in results between the human and rhesus monkey is not known.

Arachidonic acid itself affects the secretion of placental lactogen, prolactin, and insulin from the placenta (Zeitler and Handwerger, 1985), cloned rat anterior pituitary cells (Kolesnick *et al.*, 1984), and pancreatic β-cells (Metz *et al.*, 1987), respectively, independent of both lipoxygenase and cyclooxygenase pathways. Arachidonic acid's effects on placental cells might be mediated by activation of phospholipase C because addition of arachidonic acid to the medium caused the production of inositol phosphates in the cells (Zeitler and Handwerger, 1985). Arachidonic acid also stimulates guanylate cyclase (Gerzer *et al.*, 1986) and protein kinase C (McPhail *et al.*, 1984) and causes the release of calcium from intracellular stores (Wolf *et al.*, 1986).

Estrogen. The effects of estrogen on prostaglandin synthesis have been studied for the most part with uterine tissues from nonpregnant subjects. Estradiol treatment increases the uterine content and/or release of $PGF_{2\alpha}$ in several species (Blatchley *et al.*, 1971; Caldwell *et al.*, 1972; Ham *et al.*, 1975; see Horton and Poyser, 1976, for additional references). Estrogen, added to human endometrial explants in organ culture, stimulated the output of $PGF_{2\alpha}$ (Abel and Baird, 1980; Leaver and Richmond, 1984; Schatz *et al.*, 1984). The effects of estrogen on human endometrium were primarily on glandular epithelium and not on stromal cells (Schatz *et al.*, 1987).

The parallel rise in the concentration of estrone and PGFM in amniotic fluid of rhesus monkeys before spontaneous vaginal delivery (Mitchell *et al.*, 1976b) led to speculation that the increasing estrogen concentations in amniotic fluid promote production of prostaglandins

by decidual tissue. Intrafetal i. .sion of ACTH to rhesus monkeys at ab... 130 days of gestation stimulated increases in the concentrations of estrogens and prostaglandins, particularly PGE, in amniotic fluid (Novy, 1983).

Estrogen-induced increases in prostaglandin production appear to be associated with the prostaglandin synthase complex and not with the availability of arachidonic acid for prostaglandin synthesis. Administration of estrogen to guinea pigs (Wlodawer *et al.*, 1976) and rats (Ham *et al.*, 1975) increased the conversion of exogenous arachidonic acid to $PGF_{2\alpha}$ by uterine microsomes. Estrogen may have a general effect not limited to reproductive tissues. Estrogen increased the concentration of fatty acid cyclooxygenase, measured immunologically, in cultures of rat aortic smooth muscle cells (Chang *et al.*, 1983). Estrogen administration affected neither the uptake of [^{3}H]arachidonic acid by human glandular epithelial cells in cultures nor the rate of metabolism of [^{3}H]$PGF_{2\alpha}$ added to the cells (Schatz *et al.*, 1987).

Progesterone. Progesterone sometimes supported estrogen-induced increases in prostaglandin output, but in other studies progesterone antagonized the effects of estrogen (Abel and Baird, 1980). Isoxazol, an inhibitor of 3β-hydroxysteroid dehydrogenase, given to rats in late pregnancy decreased plasma progesterone and resulted in premature delivery (Csapo and Resch, 1979). At the same time, plasma estradiol and PGF levels increased. These increases were blocked by progesterone and isoxazol. Similar findings have been reported in pregnant sheep. Inhibition of 3β-hydoxysteroid dehydrogenase activity in pregnant sheep decreased placental progesterone output and led to a rapid rise in PGFM concentrations in peripheral blood and to premature delivery in most animals (Taylor *et al.*, 1982).

Khan-Dawood and Dawood (1984) found that term human decidua and myometrium had nuclear receptors for both estradiol and progesterone. Placenta, amnion, and chorion had only nuclear estrogen receptors, indicating that progesterone's effects on prostaglandin generation are more likely to be exerted on decidua than on amnion.

Calcium. Free calcium was suggested to be a major factor influencing prostaglandin output by fetal membranes (Bleasdale *et al.*, 1983). The calcium ionophore A23187 increased $PGF_{2\alpha}$ output by guinea pig (Leaver and Seawright, 1982) and human (Leaver and Richmond, 1984) endometrial explants. Because the addition of arachidonic acid also stimulated prostaglandin output, increases in intracellular ionized calcium concentrations may result in liberation of arachidonic acid from phospholipid stores. Indeed, both phospholipases A_2 and C were stimulated by Ca^{2+} *in vitro,* as was diacylglycerol lipase, which catalyzes the conversion of diacylglycerol to monoacylglycerol and frees arachidonic acid (Fig. 3). In contrast, Ca^{2+} inhibited enzymes involved in reducing arachidonic acid levels, such as diacylglycerol kinase, catalyzing the conversion of diacylglycerol to the glycerophospholipid precursor phosphatidic acid (Bleasdale *et al.*, 1983). The action of this kinase inhibits the release of arachidonic acid from diacylglycerols by recycling the diacylglycerols to phospholipids.

The effects of Ca^{2+} were clear, but they occurred at concentrations 1000 times greater than intracellular levels. Therefore, the role of intracellular changes in free calcium ion concentrations in modifying phospholipase activity is not clear. Calcium may activate protein kinase C, resulting in phosphorylation of lipocortin and the subsequent release from inhibition of phospholipase A_2 activity (see Section 4.1.5b). Phorbol 12-myristate 13-acetate (TPA), an activator of protein kinase C, stimulated $PGF_{2\alpha}$ synthesis by cultured human endometrial cells (Skinner *et al.*, 1984). However, TPA had no effect on $PGF_{2\alpha}$ production by guinea pig endometrium (Riley and Poyser, 1987), indicating that species differences exist. In guinea pig endometrium, calmodulin inhibitors prevented the stimulation of phospholipase A_2 (Riley and Poyser, 1987). The oputput of $PGF_{2\alpha}$ from guinea pig endometrium was reduced significantly by the use of Ca^{2+}-depleted medium, calcium chelator (EGTA), and an intracellular Ca^{2+} antagonist (Riley and Poyser, 1987). Calcium channel blockers also inhibited $PGF_{2\alpha}$ output. These findings indicate that extracellular Ca^{2+} is required for the high output of $PGF_{2\alpha}$ from the guinea pig uterus after day 11 of the estrous cycle

The removal of Ca^{2+} from the incubation medium reduced the output of PGE and PGF

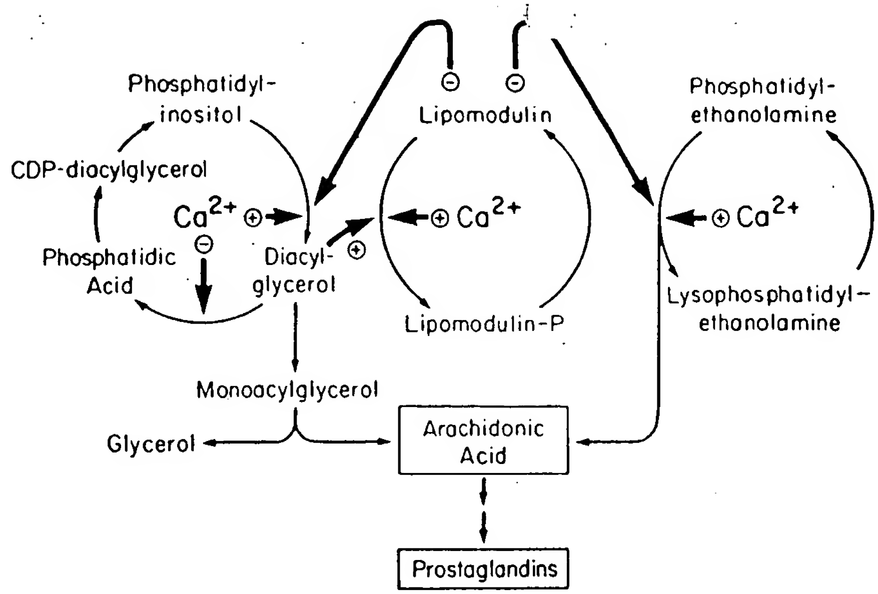

Figure 3. Proposed regulation of arachidonic acid metabolism in human fetal membranes by Ca^{2+}. From Bleasdale *et al.* (1983).

by collagenase-dispersed human amnion cells (Olson *et al.*, 1983b). Prostaglandin release also was inhibited by addition of methoxyverapamil (D-600), a calcium channel blocker. Conversely, addition of the calcium ionophore A23187 to cells was stimulatory. These results suggest that prostaglandin output by the human amnion is dependent on the entry of Ca^{2+} from amniotic fluid.

Platelet-activating factor. Billah and Johnston (1983) reported that platelet-activating factor (1-O-alkyl-2-acetyl-3-glycerophosphocholine; PAF) was present in amniotic fluid obtained from about 50% of women after active labor but was absent in fluid obtained before labor. The PAF was shown to increase cytosolic Ca^{2+} concentrations in a number of systems (see Billah *et al.*, 1985, for references). Billah *et al.* (1985) reasoned that PAF in amniotic fluid might stimulate prostaglandin synthesis by amnion cells in a calcium-dependent manner because the enzymes involved in arachidonic formation are Ca^{2+} dependent. They found that addition of PAF to amnionic tissue disks resulted in almost a threefold increase in the release of PGE_2 into the incubation medium. A similar stimulation was seen after the addition of Ca^{2+} and the calcium ionophore A23187.

Nishihara and colleagues (1984) found that PAF induced contractions of the rat isolated uterus. Similar findings were obtained with guinea pig (Montrucchio *et al.*, 1986) and human (Tetta *et al.*, 1986) myometrial strips. The response to PAF was distinct from that to oxytocin. Cyclooxygenase and lipoxygenase inhibitors blocked the PAF effect (Montrucchio *et al.*, 1986; Tetta *et al.*, 1986). In addition, desensitization to PAF but not to oxytocin occurred with a second dose. The relationship between PAF activation of prostaglandin production in amniotic fluid and its direct effects on stimulation of uterine contractions remain to be determined.

Oxytocin. Oxytocin stimulates the production of prostaglandins by endometrial and decidual tissues. This topic is elaborated on in Section 4.2.

Catecholamines and cAMP. Isoproterenol added to human amnionic disks maintained *in vitro* caused a sustained release of arachidonic acid and PGE_2 (Di Renzo *et al.*, 1984a). The effects of isoproterenol appeared to be the result of β-adrenergic activation, because dibutyryl cAMP stimulated the release of PGE_2. Similar results were obtained with other activators of adenylate cyclase including cholera toxin, forskolin, and several β agonists (Warrick *et al.*,

1985). These agents increased both cAMP production and the release of PGE and PGF from cells isolated from amnion and decidua obtained from women following spontaneous labor (Warrick *et al.*, 1985). The mechanisms of cAMP stimulation of prostaglandin synthesis are not known. It has been suggested that the catalytic subunit of cAMP-dependent protein kinase can inhibit protein kinase C-dependent phosphorylation of lipocortin in thymocytes (Hirata *et al.*, 1984). Apparently cAMP acts by different mechanisms on amnion cells, because inhibition of lipocortin phosphorylation represses prostaglandin synthesis rather than stimulates it.

During late pregnancy, the concentration of catecholamines in amniotic fluid increases (Divers *et al.*, 1981). β-Adrenoreceptors of the β_2 subtype were associated with amnionic cells and increased threefold between midtrimester and late gestation (Di Renzo *et al.*, 1984b). Increases in both agonists and receptors suggest that there would be an increased responsiveness of amnionic cells to catecholamines, resulting in increased PGE production.

Physical stimulation and tissue damage. Amniotic fluid concentrations of PGF and PGFM were significantly higher in samples obtained by amniotomy (surgical rupture of the fetal membranes) than by amniocentesis (Mitchell *et al.*, 1975b). Similarly, amniotomy or vaginal examination with sweeping of the fetal membranes caused increases in circulating PGFM levels in women studied after the 37th week of pregnancy (Mitchell *et al.*, 1977). Because labor often can be induced near term by sweeping or rupture of the membranes, some investigators consider fetal membranes to be the site of prostaglandin release. Labor usually starts, however, with intact membranes.

4.1.5b. Inhibitory Factors. *Endogenous inhibitors of prostaglandin synthesis.* Several lines of evidence suggest that prostaglandin production is tonically inhibited during pregnancy, leading some investigators to postulate that the mechanism of parturition includes withdrawal of this inhibition. Maathuis and Kelly (1978) showed that the concentration of prostaglandins in human decidual tissue obtained at 3 to 10 weeks of gestation was lower than that measured in the endometrium at any stage in the normal menstrual cycle. During the proliferative stages, the concentration of PGF in the endometrium was correlated with plasma estradiol levels. The endometrial concentration of PGE did not show any cyclic variation. The reduced level of prostaglandins in the endometrium in early pregnancy suggested that the conceptus blocked the synthesis or increased catabolism of prostaglandins. Subsequent studies showed that decidual $PGF_{2\alpha}$ and PGE concentrations in women with ectopic pregnancies were comparable to those from women with intrauterine pregnancies of the same gestational age (Abel *et al.*, 1980). Such findings suggest that suppression of endometrial prostaglandin synthesis during pregnancy may be regulated systemically instead of through a local action of the conceptus.

Saeed *et al.* (1977) demonstrated that plasma from several mammalian species inhibited the conversion of arachidonic acid to prostaglandins by homogenates of bovine seminal vesicles. This activity was ascribed to endogenous inhibitors of prostaglandin synthesis (EIPS) in Cohn fraction IV-4 of human plasma; EIPS activity also was found in human amniotic fluid (Saeed *et al.*, 1982). Inhibitory activity was greater in amniotic fluid taken in early pregnancy than in fluid taken at term but before the onset of labor. There was a further significant reduction in inhibitory activity in amniotic fluid collected during labor. These results suggested that the onset of labor was associated with the local withdrawal of inhibition of prostaglandin synthesis. It is possible that part of the inhibition was by albumin, because inhibition of prostaglandin synthesis in early and term gestation was proportional to the concentration of albumin in amniotic fluid (Saeed *et al.*, 1982). There was no correlation, however, in amniotic fluid obtained after labor.

Manzai and Liggins (1984) reported that dispersed amnionic cells released substances into the incubation medium that reduced by about 30% the output of PGE and PGF by human endometrial cells. The inhibitory activity was associated only with cells obtained from women near term before labor, not from women in spontaneous labor. Romero *et al.* (1987) reported

the presence of soluble products from human decidua that inhibited prostaglandin production by human amnion.

Brennecke *et al.* (1984) measured human maternal plasma EIPS levels during pregnancy, labor, and the puerperium. In contrast to amniotic fluid, they found no significant trends in maternal plasma levels of EIPS in relation to pregnancy and parturition. In other studies, a small but significant increase in EIPS activity was found in plasmas from women in the third trimester and at term, but this level was not maintained in labor (Fig. 4). Maternal peripheral plasma EIPS activity could not be responsible for the striking suppression of endometrial prostaglandin synthesis in early pregnancy, since there was no significant increase in plasma EIPS levels compared with values in nonpregnant women (Brennecke *et al.*, 1982). These results do not support a role for maternal plasma EIPS in the control of prostaglandin production during human pregnancy or parturition. The mechanisms that so effectively inhibit decidual prostaglandin synthesis in human pregnancy remains to be clarified.

Lipocortin. Lipocortins, a family of steroid-inducible proteins that inhibit phospholipase A_2 activity and the formation of arachidonic acid, have been isolated from various tissues and cells, including lung (Flower and Blackwell, 1979), thymocytes (Hirata *et al.*, 1984), and platelets (Touqui *et al.*, 1986). Using the amino acid sequence obtained from purified rat lipocortin, Wallner *et al.* (1986) cloned human lipocortin cDNA and expressed the gene in *E. coli*. Lipocortin produced in this way was a potent inhibitor of phospholipase A_2 activity.

Enhanced phosphorylation of lipocortin by calcium-stimulated protein kinases suppressed lipocortin inhibition of PLA_2 activity in thymocytes treated with mitogens (Hirata *et al.*, 1984) and in platelets treated with thrombin or phorbol esters (Touqui *et al.*, 1986). Lipocortins may inhibit prostaglandin production by uterine and intrauterine tissues during pregnancy, but definitive studies remain to be carried out.

4.2. *Oxytocin*

Oxytocin was considered to be the sole physiological initiator of labor because (1) it is the most potent natural substance stimulating labor, (2) the frequency and amplitude of oxytocin-

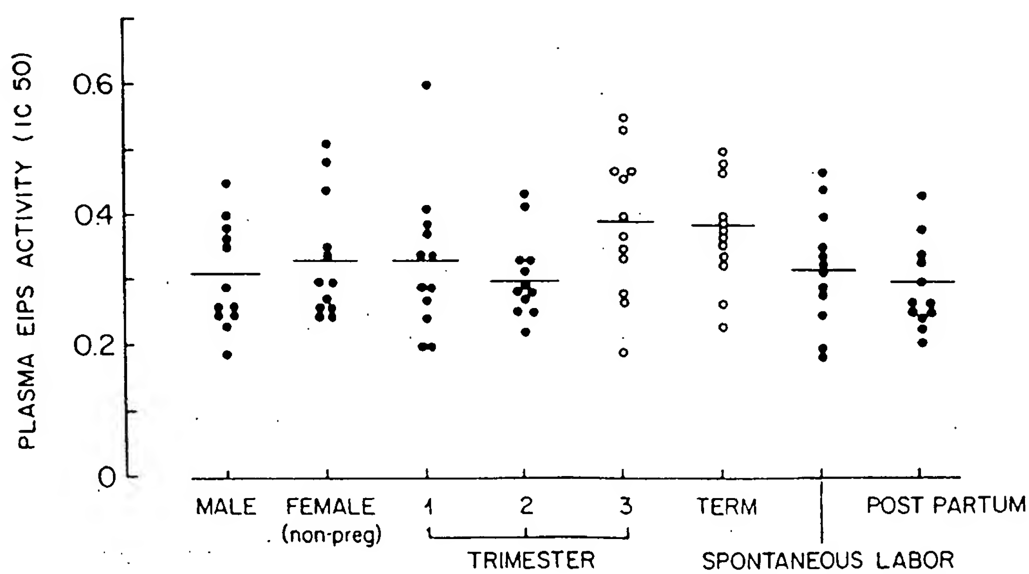

Figure 4. EIPS activities in plasmas from human subjects. The third-trimester and term groups (○) had significantly different ($P < 0.05$) activities from the first- and second-trimester and postpartum groups. From Mitchell *et al.* (1983).

induced uterine contractions are identical with those occurring during spontaneous labor, and (3) labor contractions could be induced by electrical stimulation of the posterior pituitary gland, increasing oxytocin concentrations in the blood.

Early attempts to ablate oxytocin-producing cells gave mixed results. In retrospect, the wide distribution of oxytocin-containing neurons in the magnocellular system explains why production of lesions of the posterior pituitary gland did not entirely eliminate oxytocin. Interruption of the hypothalamoneurohypophyseal tract by precise surgical lesions in the hypothalamus caused prolonged unproductive labor with a high incidence of maternal and fetal deaths in cats and guinea pigs (Dey *et al.*, 1941; Fisher *et al.*, 1938).

With the development of sensitive radioimmunoassays for oxytocin, its role in labor initiation was reassessed based on the inability to show a consistent rise in oxytocin concentrations in the peripheral maternal circulation preceding labor. Furthermore, uterine activity did not reflect oxytocin concentrations in the blood. Some proposed that maternal oxytocin levels are less significant than myometrial oxytocin concentrations arising from the fetal circulation (Fuchs and Fuchs, 1984). This concept has not been widely accepted.

During the expulsive stage of labor, there is a substantial rise in oxytocin concentrations in the maternal circulation (Leake *et al.*, 1981), leading to the conclusion that, although oxytocin may not initiate labor, the release of oxytocin during labor results in more forceful uterine contractions, facilitating delivery of the baby and placenta. The stimulation of oxytocin release during the expulsive phase of labor has been attributed to the Ferguson reflex following cervical and vaginal distension by the emerging baby (Ferguson, 1941).

Oxytocin was displaced as a candidate for the initiator of labor by the almost universal acceptance of the importance of prostaglandins. Present evidence suggests that oxytocin is involved in labor initiation, perhaps alone but probably in conjunction with prostaglandins. This conclusion is based on the following observations. First, the myometrium of all species that have been studied is most sensitive to oxytocin either near or at the time of labor. The sensitivity changes appear to result, at least in part, from increases in myometrial oxytocin receptor concentrations. Second, oxytocin receptors have been demonstrated in endometrium and decidua, and oxytocin is capable of stimulating prostaglandin synthesis in these tissues. The elevation of prostaglandin levels makes the myometrium more sensitive to oxytocin. Third, an oxytocin antagonist inhibits uterine contractions of premature labor. Teleologically, oxytocin must be important to the organism inasmuch as no oxytocin-deficient states have yet been described.

4.2.1. *Sensitivity of the Myometrium to Oxytocin*

In all species that have been studied, maximal myometrial sensitivity to oxytocin occurs at or near the time of labor (Fig. 5). More than 100 mU of oxytocin infused per minute is needed to elicit uterine contractions in nonpregnant women, whereas 16 mU/min is sufficient to elicit contractions at 20 weeks of pregnancy, 2 mU/min at 32 weeks, and 1 mU/min at term (Caldeyro-Barcia and Sereno, 1961). Theobald (1959) suggested that labor commences when the uterus becomes sufficiently sensitive to circulating oxytocin. However, this suggestion was largely ignored, and plasma oxytocin levels were given greater emphasis.

Support for Theobald's notion has come from Takahashi *et al.* (1980), who performed weekly oxytocin challenge tests on a group of high-risk patients. They found retrospectively that women who would give birth prematurely responded to lower doses of oxytocin than did those at the same time of gestation who would go to full term. Women with gestational length beyond term required the highest doses of oxytocin. At delivery, irrespective of the length of gestation, the uteri of preterm, postterm, and normal-term patients had similar sensitivities to oxytocin.

The enhanced uterine sensitivity to oxytocin appears to be largely a consequence of an increase in the effective concentration of oxytocin receptors on myometrial plasma mem-

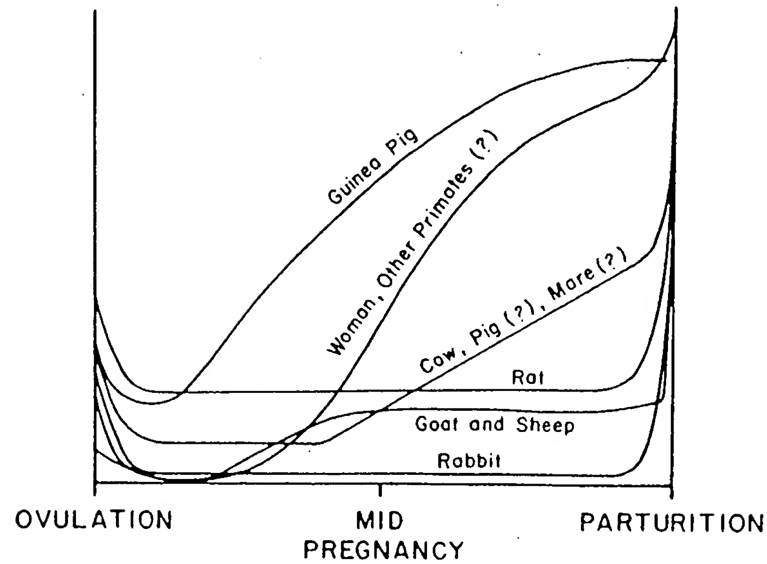

Figure 5. Changes in uterine sensitivity to oxytocin in various species, based on publications by different authors. From Fuchs (1985).

branes. In the rat, exogenous oxytocin initiates parturition only 1 day or less before the time of labor (Fuchs and Poblete, 1970), when there is a sharp rise in the concentration of myometrial oxytocin receptors (Fig. 2). The concentration of receptors is highest at labor and then falls abruptly to preterm levels 1 or 2 days after parturition (Alexandrova and Soloff, 1980a; Soloff *et al.*, 1979). When labor is induced 2 or 3 days early with $PGF_{2\alpha}$ there is a premature rise in oxytocin receptor concentrations in the myometrium (Alexandrova and Soloff, 1980b). If labor is delayed by 1 or 2 days by pharmacological amounts of LHRH, there is a similar delay in the increase in myometrial oxytocin receptor concentrations (Bercu *et al.*, 1980).

Therefore, an increased oxytocin concentration in the blood may not be necessary to stimulate myometrial cells; an increase in the number of oxytocin receptors allows myometrial cells to respond to basal levels of oxytocin.

4.2.2. *Oxytocin Receptors*

4.2.2a. Relationship between Oxytocin Receptor Concentration and Sensitivity. The relationship between the concentration of myometrial oxytocin receptors and the sensitivity of the myometrium to oxytocin *in vivo* was studied in individual pregnant rats (Fuchs *et al.*, 1983a). The animals, with intrauterine balloons to monitor uterine contractions, were given intravenous infusions on days 20, 21, 22, or 23 of gestation of increasing concentrations of oxytocin. After the threshold dose of oxytocin was ascertained, oxytocin receptor concentrations in myometrial plasma membranes were measured. A significant correlation was found between the concentration of oxytocin receptors and the sensitivity of the uterus to oxytocin.

Parturition in the rat is followed by a sharp decline in the concentration of myometrial oxytocin receptors. The marked reduction in uterine sensitivity to increased concentrations of oxytocin in the blood resulting from milk-ejection stimuli results from down-regulation of receptors. In the absence of other changes, a reduction in membrane receptors decreases target cell sensitivity in tissues containing abundant spare receptors and decreases target cell response when receptor concentration is limited.

Changes in receptor concentrations in guinea pig (Alexandrova and Soloff, 1980c) and human (Fuchs *et al.*, 1982) myometrial plasma membranes also corresponded to well-estab-

lished oxytocin sensitivities. In women in labor, the number of myometrial oxytocin receptors per milligram of DNA was more than 150 times greater than that in nonpregnant women (Fuchs *et al.*, 1982, 1984). The timing of the increase in sensitivity corresponds to the time of increase in oxytocin receptor concentrations. Oxytocin receptor concentrations doubled at the onset of labor (cervix dilated less than 4 cm). Women at term or beyond but not responsive to oxytocin had relatively low oxytocin receptor levels. Women in preterm labor had elevated receptor concentrations comparable to those of women in term labor. The concentration of myometrial oxytocin receptors increases abruptly on the last day of gestation in rabbits (Riemer *et al.*, 1986) and corresponds to the time of the increased sensitivity to oxytocin in this species (Caldeyro-Barcia and Sereno, 1961).

An apparent exception to the relationship between receptor number and sensitivity was the observation that estrogen-treated Brattleboro rats had 15% the receptor concentration of myometrial membranes from similarly treated Sprague–Dawley rats (Goren *et al.*, 1980). Despite this difference, the uterine contractile response to oxytocin *in vitro* was the same in both strains. However, Haldar and co-workers (1982) found that uteri from estrogen-treated Brattleboro rats had about 25% the sensitivity to oxytocin as did uteri from Long–Evans rats, a strain more closely related to Brattleboro rats than the Sprague–Dawley strain. Similar results were obtained when oxytocic activity was measured either *in vitro* or *in vivo*.

Crankshaw (1987) surmised that changes in the number of receptor sites for oxytocin in uterine smooth muscle are not important for the changes in oxytocin sensitivity. Using longitudinally cut strips from rat uteri, he found that there was no significant change in the dose of oxytocin giving half-maximal stimulation *in vitro* with advancing gestation. A considerable number of strips responded to oxytocin in early and midgestation, when binding sites for oxytocin are relatively low. Circularly cut muscle strips, however, were essentially refractory to oxytocin until day 21 of pregnancy, suggesting that oxytocin receptor concentrations might be important in regulating the response to oxytocin only in circular smooth muscle. Not only were Crankshaw's findings in disagreement with *in vivo* data (Fuchs and Poblete, 1970), but they also did not concur with the observations of Kuriyama and Suzuki (1976) that the threshold dose of oxytocin required to stimulate electrical activity in longitudinally cut strips of rat myometrium declined sharply at the end of gestation.

Interpretations of experiments with cut muscle strips *in vitro* should be taken with caution. Riemer *et al.* (1986) found a nearly tenfold increase in the concentration of oxytocin receptors in the rabbit myometrium between days 30 and 31 (term) of gestation, along with at least a fourfold oxytocin sensitivity increase *in vitro*. The lesser sensitivity to oxytocin on day 30, however, was apparent only in the presence of meclofenamate, an inhibitor of eicosanoid formation. Because the rabbit myometrium *in vivo* becomes abruptly sensitive to oxytocin only at the end of gestation (Caldeyro-Barcia and Sereno, 1961), the release of prostaglandins *in vitro*, probably as the result of tissue damage, obliterated the shift in oxytocin sensitivity between days 30 and 31. There is evidence to suggest, however, that endogenous prostaglandins, released perhaps in response to oxytocin stimulation, may play an important part in modulating oxytocin action *in vivo*. Notwithstanding the possibility that regulation of sensitivity to oxytocin *in vivo* may occur at sites beyond oxytocin–receptor interaction, data from several species are all consistent with regulation occurring at the receptor level.

4.2.2b. Topographical Distribution of Oxytocin Receptors. The localization of oxytocin receptors in the human myometrium corresponds to the directionality of uterine contractions during labor. The concentration of receptors in plama membrane fractions from uterine fundus and corpus were significantly higher than the concentration from the isthmus or ampulla of fallopian tubes (Fuchs *et al.*, 1984, 1985). The lowest concentration of oxytocin binding sites per cell (milligram of DNA) was found in cervical plasma membranes. The topographical distribution of receptor sites appears to follow the relative content of smooth muscle cells (Schwalm and Dubrauszky, 1966). Similar results have been obtained with [^{3}H-

PGE_1 and [^{3}H]PGE_2 binding sites in crude membrane preparations from nonpregnant human myometrium (Hofmann *et al.*, 1983). These findings may explain why during the first stage of labor the work of the fundus exceeds that of any other part of the uterus, while the lower uterine segment is inactive (Reynolds *et al.*, 1948). Parenthetically, the distinct topographical distribution of oxytocin receptors suggests that care be taken in ensuring that myometrial samples are obtained from the same region of the uterus.

4.2.2c. Regulation of Oxytocin Receptor Concentrations. It is not yet clear whether increases in oxytocin binding at the end of gestation are the result of increased numbers of receptors per cell, increased numbers of cells containing oxytocin receptors, or both. This question probably can be answered most directly by autoradiographic techniques, which also could define whether the longitudinal, circular, or both layers of the myometrium undergo changes in receptor number during pregnancy.

The molecular basis for the increase in receptor number is also unclear. Possibilities include the *de novo* synthesis of receptors, unmasking of cryptic receptor sites, activation of existing receptor sites by mechanisms such as phosphorylation/dephosphorylation, conversion of a precursor to an active protein, or the appearance/disappearance of activating/inhibiting substances. Understanding of the mechanisms of up-regulation of oxytocin receptors will be facilitated by their purification or the development of specific antireceptor antibodies.

Factors involved in up- and down-regulation of oxytocin receptors. Parturition in the rat appears to occur as a result of a fall in progesterone and a rise in estrogen levels in the blood. These changes are correlated with increases in the concentration of myometrial oxytocin receptors (Alexandrova and Soloff, 1980a; Soloff *et al.*, 1979). Induction of premature labor (Alexandrova and Soloff, 1980b) or delay of parturition (Bercu *et al.*, 1980) in rats is accompanied by premature or delayed increases, respectively, in both plasma estrogen/progesterone ratios and oxytocin receptor concentrations.

The effects of estrogen and progesterone on oxytocin receptor concentrations in rat myometrium have been demonstrated both *in vivo* (Fuchs *et al.*, 1983d) and *in vitro* (Fig. 6). Estrogen treatment increased oxytocin receptor concentrations about five-fold; this increase was completely blocked by progesterone. On the basis of these findings, changes in oxytocin receptor concentrations may explain why estrogen administered at the appropriate time of gestation causes abortion in the rat and several other species and why progesterone administration prolongs pregnancy.

Estrogen treatment of women at 40 to 42 weeks of pregnancy potentiated the effects of a single dose of oxytocin by increasing the intensity of uterine contractions (Pinto *et al.*, 1964). It is not clear, however, whether these effects were mediated by increases in oxytocin receptor concentrations.

Mechanisms of estrogen and progesterone regulation of oxytocin receptors. Estrogen and progesterone act directly on immature rat uteri in organ culture (Soloff *et al.*, 1983). The protein synthesis inhibitor cycloheximide in the culture medium prevented estrogen-induced increases in oxytocin receptor concentrations. Estrogen, therefore, may induce *de novo* synthesis of oxytocin receptors or the synthesis of substances that enhance oxytocin binding to its receptors. When cycloheximide was added after estrogen-induced up-regulation of oxytocin receptors, receptor concentrations remained elevated for at least several days, indicating that there was little or no turnover of receptors. When progesterone was also added, however, there was a sharp reduction in the amount of oxytocin bound. These results suggest that the down-regulating effects of progesterone are distinct from its antiestrogenic activity, because estrogen action was presumably already antagonized by the presence of cycloheximide. Progesterone, therefore, appears to down-regulate oxytocin receptors by pathways that are not the reverse of up-regulation.

Metal ions. Magnesium directly enhances the contractile activities of oxytocin and analogues in uterine smooth muscle, mammary myoepithelial, and vascular smooth muscle

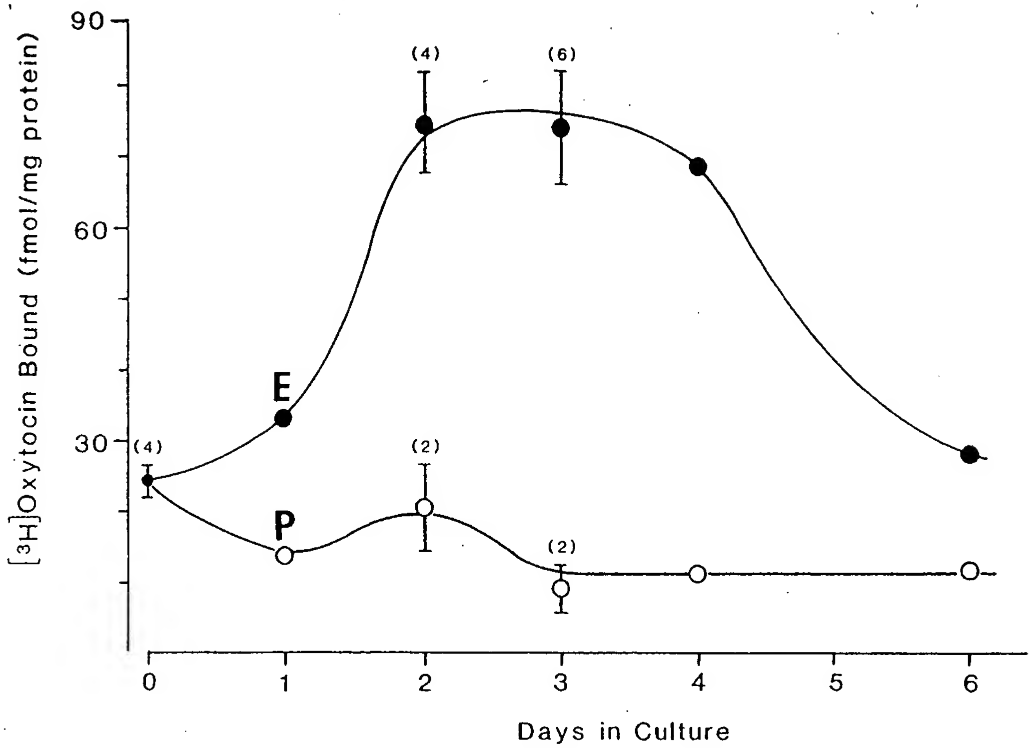

Figure 6. Effects of estradiol (E) and progesterone (P) *in vitro* on the concentration of oxytocin receptors in uterine explants from immature rats. Each point is the mean ± S.E. of *n* replicates. From Soloff *et al.* (1983).

cells (see Soloff and Grzonka, 1986, for references). Other contracting agents are not affected by Mg^{2+}. Certain divalent cations, but not Ca^{2+}, are required for the binding of [^{3}H]oxytocin to both myometrial (Soloff and Grzonka, 1986; Soloff and Swartz, 1974) and mammary gland (Pearlmutter and Soloff, 1979; Soloff and Grzonka, 1986) plasma membranes. The effects of metal ions in potentiating the activities of oxytocin and its analogues are at the receptor level (Soloff and Grzonka, 1986). Metal ions may be important in the up- and down-regulation of oxytocin receptor concentrations and for the coupling between oxytocin receptors and effectors.

4.2.3. Coupling of Oxytocin–Receptor Occupancy and Contraction

4.2.3a. Calcium. Extracellular Ca^{2+} is involved in the interaction with calmodulin, leading to phosphorylation of myosin light chains and cell contractions. Voltage-clamp studies suggest that an increased Ca^{2+} permeability explains at least some of the inward current during electrical depolarization of the myometrium (Janis and Triggle, 1986). The generation of an action potential, however, does not appear to mediate the effects of many agonists such as acetylcholine (Edman and Schild, 1962) and oxytocin (Marshall, 1974), which cause Ca^{2+}-dependent contractions of the rat myometrium even after K^+-induced depolarization.

4.2.3b. Calcium Channels. Dihydropyridine calcium channel blockers inhibit spontaneous uterine contractions and contractions induced by oxytocin, $PGF_{2\alpha}$, and methylergometrine (Forman *et al.*, 1982). There are functionally distinct Ca^{2+} channels in estrogen-dominated myometrium (Sakai *et al.*, 1983). Channels mediating the action of oxytocin transport Mn^{2+} as well as Ca^{2+}, whereas Ca^{2+} channels activated by acetylcholine are specific for Ca^{2+} and impermeable to Mn^{2+} (Sakai *et al.*, 1983).

Nicardipine, a dihydropyridine blocker, protracted delivery of pups when given to par-

turient rats immediately after birth of the first pup (Csapo *et al.*, 1982). Nifedipine, another dihydropyridine blocker, postponed premature labor in humans for 3 days without serious side effects in either mother or child (Ulmsten *et al.*, 1980).

The characteristics of [^{3}H]nitrendipine and [^{3}H]nimodipine binding to uterine membranes and other smooth muscle membranes are similar. A high-affinity binding site, apparent K_d about 0.1 nM, is present in rat and rabbit myometrium (Grover *et al.*, 1984; Miller and Moore, 1984; Golichowski and Tzeng, 1985; Janis and Triggle, 1986). Neither estrogen treatment of rats nor pregnancy produced any marked change in the affinity (Janis and Triggle, 1986). Similarly, the concentration of binding sites was not significantly changed between day 1 of pregnancy and term (Janis and Triggle, 1986). The appearance of Ca^{2+} channel binding sites therefore does not appear to be linked to the increase of oxytocin receptors near the time of labor. Uterine contractions caused by relatively low concentrations of oxytocin are inhibited more by Ca^{2+} channel blockers than are contractions induced by higher concentrations of oxytocin (Janis and Triggle, 1986). The coupling between agonist receptors and calcium channels has not yet been defined.

4.2.3c. Oxytocin-Inhibited (Ca^{2+}, Mg^{2+})-ATPase. Relaxation of myometrial cells is thought to occur when intracellular free Ca^{2+} concentrations decline. This can be brought about by a switch in sarcolemmal Ca^{2+} channels from an open to a closed state or by an increased rate of efflux of Ca^{2+} from the cytosol. If myometrial cells are similar to other cell types, the efflux of Ca^{2+} is controlled by a plasma-membrane calcium pump, which has been shown in a variety of cells to exhibit (Ca^{2+}, Mg^{2+})-ATPase activity (Gietzen *et al.*, 1980). Oxytocin inhibits (Ca^{2+}, Mg^{2+})-ATPase activity in the plasma membrane fraction of myometrium from the rabbit (Åkerman and Wikström, 1979) and rat (Soloff and Sweet, 1982). By inhibiting the efflux of Ca^{2+}, oxytocin allows a transient rise in intracellular Ca^{2+} concentration to remain elevated longer, sustaining a contractile state. The inhibition of the ATPase by a series of oxytocin analogues corresponded to their ability to inhibit the binding of [^{3}H]oxytocin and to their relative uterotonic potencies (Soloff and Sweet, 1982). Concentrations of oxytocin for half-maximal inhibition of (Ca^{2+}, Mg^{2+})-ATPase activity also corresponded to the apparent K_d for oxytocin binding to its receptor sites (Soloff and Sweet, 1980). Oxytocin receptors and oxytocin-inhibited (Ca^{2+}, Mg^{2+})-ATPase in rat myometrium were induced by estrogen treatment and inhibited by progesterone (Soloff and Sweet, 1980). At the beginning of labor in the rat, the suppressibility of myometrial (Ca^{2+}, Mg^{2+})-ATPase activity was increased about 10,000-fold, as compared to the inhibition on day 18 (Fig. 7). These changes probably are the result of the sudden shift to estrogen domination at the time of labor and reflect sharp increases in both oxytocin receptor concentations and basal (Ca^{2+}, Mg^{2+})-ATPase activity.

4.2.3d. Phosphoinositol Metabolism. Several studies have suggested that the effects of oxytocin on the myometrium are mediated by polyphosphoinositide hydrolysis. Marc *et al.* (1986), using guinea pig myometrial strips that were prelabeled with [^{3}H]*myo*-inositol, found that both carbachol and oxytocin enhanced the rapid formation of inositol trisphosphate. Similarly, oxytocin and vasopressin stimulated the production of inositol phosphates in human gestational myometrium and decidua (Schrey *et al.*, 1986). These findings, along with those of Carsten and Miller (1985), who showed that inositol trisphosphate caused release of Ca^{2+} from sarcoplasmic reticulum vesicles from the myometrium of pregnant cows, suggest that phosphoinositol formation could mediate the actions of oxytocin in elevating intracellular Ca^{2+} concentrations from intracellular stores as well as from the exterior. Studies on the stimulatory activities of a series of oxytocin analogues remain to be done to show whether the effects of oxytocin on phosphoinositol hydrolysis are mediated by oxytocin and not vasopressin receptors. Vasopressin has been shown to stimulate phosphosinositol degradation in the vasculature (Fox *et al.*, 1987), and it is possible that oxytocin's effects are on vascular cells in the endometrium and myometrium.

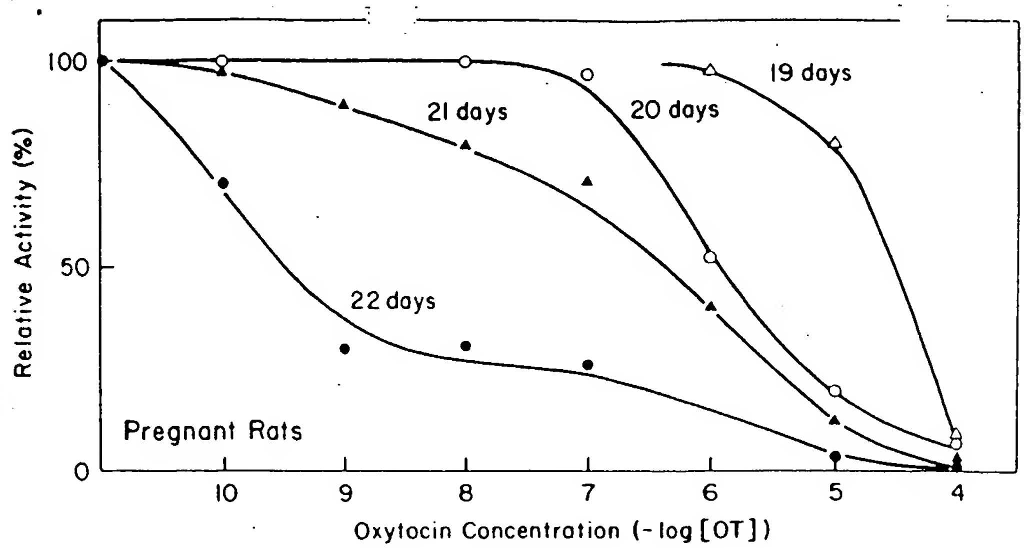

Figure 7. Oxytocin inhibition of Ca^{2+}, Mg^{2+}-ATPase activity of rat myometrial plasma membranes on days 19–22 of pregnancy. From Huszar (1986).

4.2.4. Stimulation of Endometrial/Decidual Prostaglandin Synthesis by Oxytocin

Apart from its uterotonic activity, oxytocin stimulates uterine prostaglandin release in several species (Chan, 1977; Mitchell *et al.*, 1975a; Roberts *et al.*, 1975; Sharma and Fitzpatrick, 1974). Oxytocin receptor concentrations in the plasma membrane fraction from homogenates of ewe endometrium increased at estrus, in conjunction with an increased sensitivity to oxytocin of $PGF_{2\alpha}$ release by endometrial explants (Roberts *et al.*, 1976). Several days before and after estrus, both oxytocin receptor concentrations and oxytocin stimulation of $PGF_{2\alpha}$ synthesis were substantially lower. Oxytocin is likely to play a role in luteal regression and to control estrous cyclicity in some species by stimulating the uterine synthesis of prostaglandins, which are luteolytic (McCracken *et al.*, 1972). Oxytocin, administered before or near the time of estrus, shortened the estrous cycle in heifers (Anderson *et al.*, 1965; Armstrong and Hansel, 1959). Immunization against oxytocin delayed luteolysis in sheep (Sheldrick *et al.*, 1980) and goats (Cooke and Homeida, 1985).

Oxytocin stimulated PGF and PGE release from decidual explants of patients at the end of gestation, either before or after the onset of labor (Fuchs *et al.*, 1982). Basal and oxytocin-stimulated prostaglandin production were significantly higher in decidual samples taken from patients after the onset of labor. In contrast to decidua, oxytocin had no effect on prostaglandin production by myometrial samples from the same uteri. An increase in oxytocin-stimulated prostaglandin release also occurred in the rat uterus in late pregnancy (Chan, 1977).

As in myometrium, oxytocin stimulated the formation of inositol trisphosphate in carnucular endometrium from ovariectomized ewes treated with estrogen and progestin (Flint *et al.*, 1986). Flint and co-workers (1986) proposed that stimulation of endometrial prostaglandin synthesis by oxytocin is the result of increased hydrolysis of phosphoinositides to diacylglycerol and inositol phosphates. The hydrolysis of diacylglycerol then releases arachidonic acid to serve as the substrate for the synthesis of prostaglandins.

4.2.5. Decidual Oxytocin Receptors

Concentrations of oxytocin receptors in parietal decidua increased during pregnancy and were maximal in early labor (Fuchs *et al.*, 1982, 1984). Decidual receptors had the same affinity for oxytocin as did myometrial receptors, but the binding sites have not yet been

further characterized. A relationship between receptor occupancy and stimulation of prostaglandin synthesis also has not been characterized yet, nor have the factors regulating receptor concentrations during pregnancy. Because the increases in decidual and myometrial oxytocin receptor concentrations appear to be parallel, the same factors probably regulate both receptors. It is clear from results in nonpregnant sheep that oxytocin receptor concentrations in the endometrium and the release of $PGF_{2\alpha}$ in response to oxytocin *in vitro* are greatest during estrogen domination (Roberts *et al.*, 1976).

The effects of oxytocin on prostaglandin synthesis appear to be important in labor. Husslein *et al.* (1981) found that oxytocin infusion into women at term led to successful induction of labor only when PGFM levels in the maternal circulation became elevated. These workers also found that the success rate for the induction of labor by amniotomy was associated with the uterine sensitivity to oxytocin (see Husslein *et al.*, 1983). Presumably, prostaglandins released as a result of amniotomy reached the myometrium and potentiated the action of subthreshold levels of oxytocin so that contractile activity was initiated.

4.2.6. *Relationship between Oxytocin and Prostaglandins*

4.2.6a. Prostaglandin Sensitization of Myometrium to Oxytocin. Saldana *et al.* (1974) showed that prostaglandin converted the human uterus from an oxytocin-resistant to an oxytocin-sensitive organ. They and others (Perry *et al.*, 1977; Salomy *et al.*, 1975; Seppala *et al.*, 1972) demonstrated shorter injection–abortion intervals in midtrimester patients receiving intravenous oxytocin along with prostaglandin. The mechanism of the sensitization has not been studied to date. Unlike oxytocin, endogenous prostaglandins might not act primarily as uterotonic agents because intravenous infusion of prostaglandins resulted in notably slow stimulation of uterine activity, with a latency period of 15 to 20 min and persistence of the uterotonic effect for 30 to 60 min after the end of the infusion (Embrey, 1969). Oxytocin infusions, on the other hand, result in almost immediate and sustained uterine contractions only for the duration of the infusion.

Other studies have shown that following the addition of PGE to the medium bathing either guinea pig (Clegg and Pickles, 1966) or human myometrial strips (Brummer, 1971), the subsequent response and sensitivity to oxytocin were enhanced. This effect, which did not result from the additive uterotonic properties of PGE and oxytocin, was seen with strips from human uteri taken at midtrimester and at term. Sometimes the enhancement lasted for as long as 90 min after the PGE had been washed out of the bath. Uterine contractions elicited by oxytocin *in vivo* could also be enhanced when patients were pretreated with PGE_2 (Gillespie, 1972). The enhancement, which persisted for 60 to 90 min after the end of the PGE_2 infusion, was distinct from potentiation, which occurred when oxytocin and PGE_2 were administered simultaneously. Brummer (1971) hypothesized that during the process of parturition endogenous prostaglandins, rather than having a direct action on the myometrium, sensitize the uterine smooth muscle to oxytocin. This might explain why the blood levels of prostaglandins attained during infusion for the induction of labor seem to be too low to promote uterine contractions directly.

4.2.6b. Mechanisms of Prostaglandin Enhancement of Oxytocin Action. The molecular mechanisms of prostaglandin enhancement of myometrial sensitivity to oxytocin are unknown. Prostaglandins and oxytocin, both uterotonic agents, do not act through a common pathway in eliciting uterine contractions because their effects are not additive.

Because of an earlier observation that prostaglandin synthesis inhibitors inhibited the action of oxytocin on the myometrium (Hertelendy, 1973; Vane and Williams, 1972), it was assumed that the actions of oxytocin were mediated by prostaglandins. However, several laboratories showed by different approaches that the stimulation of uterine muscle contraction

by oxytocin did not require prostaglandin release (Chan 1980; Dubin *et al.*, 1979; Roberts and McCracken, 1976). Unfortunately, these experiments were not designed to ascertain whether prostaglandins sensitized the uterus to oxytocin. The doses of indomethacin, a prostaglandin synthesis inhibitor used to inhibit the effects of oxytocin in the earlier experiments, might have inhibited calcium uptake rather than prostaglandin synthesis (Northover, 1972). A still untested possibility is that during the period between the infusion of prostaglandin and the effect on the uterus, the number of gap junctions between myometrial cells increases, allowing more cells to respond to a given dose of oxytocin. When a response to a low dose of oxytocin becomes perceptible because of the increased participation of cells, the myometrium would appear to be more sensitive to oxytocin.

Another possibility is that prostaglandins affect the number of oxytocin receptors. Prostaglandin $F_{2\alpha}$ administered to rats on day 18 of pregnancy caused a marked rise in oxytocin receptor concentrations in myometrial membranes 2 days later, in conjunction with premature delivery (Alexandrova and Soloff, 1980b). But the effect of prostaglandin may be indirect and the result of its luteolytic activity, causing a sharp fall in blood progesterone. Exogenous progesterone administration overrides the effects of the prostaglandin. Estrogen-induced increases in oxytocin receptor concentrations in ovariectomized rats are not prostaglandin mediated because concomitant treatment of the rats with indomethacin had no effect (Soloff and Alexandrova, 1981).

Apart from the pharmacological actions of prostaglandins, Chan (1987) suggested that prostaglandins stimulate physiological increases in myometrial oxytocin receptor concentrations during pregnancy. He found that suppression of prostaglandin synthesis with naproxen reduced the sensitivity of the myometrium of the rat to oxytocin in late pregnancy and reduced the concentration of myometrial oxytocin receptors. Simultaneous treatment with $PGF_{2\alpha}$ prevented the effects of naproxen. Although the actions of prostaglandins may be mediated by luteolysis in rats, the same mechanism does not occur in humans, who do not require a corpus luteum for the synthesis of progesterone near the end of gestation. The human does not depend on the demise of the corpus luteum or on progesterone withdrawal as a prerequisite for the initiation of parturition. The addition of 10 μM $PGF_{2\alpha}$ to myometrial plasma membranes of uteri from pregnant women caused about a twofold, significant increase in affinity for oxytocin (Fukai *et al.*, 1984). There was no change in the concentration of oxytocin binding sites. The $PGF_{2\alpha}$ effect was seen only with myometrial membranes obtained from patients at term but before labor and not in the first trimester or at term after labor (Fukai *et al.*, 1984). Whether the effects of $PGF_{2\alpha}$ on oxytocin binding are pharmacological or physiological remains to be established.

The concept that prostaglandins affect oxytocin receptor concentrations may explain the requirement for a latency period between the administration of prostaglandins and a uterotonic response *in vivo*. Latency may be the time required for an effective increase in the number of oxytocin receptors in the myometrium, allowing smooth muscle cells to respond to basal levels of oxytocin in the blood. The latency may also result from the establishment of postreceptor modifications.

4.2.6c. Prostaglandin-Induced Release of Oxytocin. Intravenous infusion of PGE_2 or $PGF_{2\alpha}$ increased plasma oxytocin levels in pregnant women (Gillespie *et al.*, 1972). Intramuscular injection of $PGF_{2\alpha}$ also elevated plasma oxytocin concentrations in sows both in the postpartum period and during diestrus (Ellendorff *et al.*, 1979). In addition, prostaglandins stimulated the release of oxytocin from ewe ovaries (Flint and Sheldrick, 1982). Other studies, however, showed that although oxytocin stimulates the release of uterine $PGF_{2\alpha}$ in pregnant and nonpregnant sheep, prostaglandins do not appear to affect plasma oxytocin levels (Hooper *et al.*, 1986). Although prostaglandins may enhance oxytocin action *in vivo* by stimulating an increase in oxytocin levels in the blood, it is not clear how this mechanism would account for the enhancing effects of prostaglandins on oxytocin action *in vitro*.

4.2.7. Oxytocin Antagonists

A recently synthesized competitive inhibitor of the effects of oxytocin on the uterus, 1-deamino-2-D-Tyr-(OEt)-4-Thr-8-Orn-oxytocin, has become available (Åkerlund *et al.*, 1987). Intravenous infusion of 10 to 100 μg/min for 1 to 10 hr in 13 patients exhibiting uncomplicated premature labor resulted in inhibition of uterine activity (Åkerlund *et al.*, 1987). No side effects were observed on either mother or fetus. This preliminary study supports the concept that an increased concentration of uterine oxytocin receptors is important in uncomplicated premature labor.

4.3. Relaxin

Despite the evidence for a role of relaxin in promoting myometrial quiescence, other studies have shown that the administration of relaxin near the end of gestation accelerates the time of delivery. The administration of purified porcine relaxin into the cervical os of primiparous beef heifers about 5 days before term induced premature calving (Musah *et al.*, 1986). Similarly, the administration of porcine relaxin induced labor in 10 of 30 patients 15 hr after vaginal application, whereas no patients in the control group went into labor (MacLennan *et al.*, 1980).

Downing and Sherwood (1985a) made pregnant rats relaxin-deficient by removing the ovaries and replacing estradiol and progesterone by injection. These animals exhibited significantly prolonged gestation, prolonged duration of labor and delivery, and reduced fetal survival compared with animals receiving porcine relaxin or intact controls. The parturitional effects of relaxin might may be the result of better coordination of contractions (Downing *et al.*, 1980). The mechanism of this relaxin effect is not known. Relaxin may stimulate uterine glycogen synthesis. Relaxin administered after large doses of estrogen was shown to increase myometrial glycogen content. Administration of progesterone with relaxin in estrogen-primed animals caused a further increase in uterine glycogen (Kroc *et al.*, 1959). Because of the sharp change in glycogen content in the rat myometrium near the time of labor (Chew and Rinard, 1979), glycogen serves as a likely energy source for uterine contractions in labor.

5. Cervical Distensibility

The histological structure of the cervical stroma undergoes marked changes prior to parturition in most species. This reflects the conversion of the cervix from a closed and rigid to a soft and distensible structure, permitting passage of the term fetus. Abnormalities in the cervical ripening process may result in preterm delivery if they occur too early and may be associated with postterm or prolonged labor if they fail. In the rat, cervical softening begins by day 12 of pregnancy and increases progressively throughout the remainder of pregnancy (Downing and Sherwood, 1985c). In humans, cervical maturation begins at about the 34th week of pregnancy, a time when irregular Braxton–Hicks uterine contractions are also noted.

The biochemical changes in the cervix leading to changes in its mechanical properties have been reviewed by Golichowski (1986) and Stys (1986). The bulk of normal cervix is collagen; the muscle fiber content is less than 10% of cervical bulk (Danforth, 1947). Cervical maturation is characterized by a degradation of collagen and other proteins, resulting in loosening of the compactly arranged collagen fibers, and changes in the composition of the glycosaminoglycan ground substance. A marked increase in hyaluronic acid and its associated high water content causes the soft, swollen appearance of the cervix, whereas loss of collagen

and dermatan/chondroitin sulfates increases flexibility and distensibility (Danforth *et al.*, 1974).

5.1. Hormonal Control of Cervical Maturation

Several hormones influence cervical ripening. Some have been used to promote cervical ripening before induction of labor to increase the success rate of induction and to shorten the induction-to-delivery time.

5.1.1. Estrogens

Ripening of the cervix occurs under the influence of estradiol (Pinto *et al.*, 1964). In a double-blind study, Gordon and Calder (1977) showed that extraamniotic instillation of estradiol in primigravid patients near term with unripe cervices increased the Bishop scores significantly and allowed induction of labor with greater success. The involuting uterus and explants of cervical and uterine tissues have served as experimental models for a study of the effects on collagenase activity and collagen breakdown of steroid hormones. Estrogens increase collagenase formation by synthesis and activation of zymogens. The inhibitory effect of progesterone on cervical maturation is well documented. In response to progesterone administration, collagen breakdown was diminished in the uterus of parturient rats (Tansey *et al.*, 1978) and in the guinea pig pubic symphysis ligament (Wahl *et al.*, 1977).

5.1.2. Relaxin

Relaxin is involved in the biochemical and biophysical changes in the uterine cervix that promote cervical dilatation immediately prior to parturition (for reviews see Schwabe *et al.*, 1978; Bryant-Greenwood, 1982). In rats, removal of the ovaries, the source of relaxin, during late pregnancy followed by treatment with progesterone and estrogen resulted in failure of the cervix to exhibit increased extensibility (see Downing and Sherwood, 1985c, for references). When ovariectomized pregnant rats were treated with porcine relaxin in conjunction with estrogen and progesterone, their cervices exhibited similar extensibility and an ability to accommodate to stretch as did cervices from intact pregnant rats of the same stage of late pregnancy (Downing and Sherwood, 1985c). The extensibility of the cervix of estrogen-primed mice also was increased by treatment with porcine relaxin (Fields and Larkin, 1980). In pigs, injection of relaxin induced premature cervical dilatation and reduced the delivery time (Kertiles and Anderson, 1979). In the human near term, MacLennan *et al.* (1980) have presented evidence suggesting that exogenous relaxin facilitates cervical ripening with little change in uterine activity.

Relaxin binding sites have been demonstrated in membrane preparations from the cervices of pigs treated with gonadotropins (Mercado-Simmen *et al.*, 1982). The concentration of [^{125}I]relaxin binding sites fell sharply after ovariectomy and could not be restored by estrogen treatment. In contrast, the number of myometrial membrane binding sites, which also fell after ovariectomy, increased after estrogen treatment. These findings suggest that relaxin receptors concentrations in the cervix are regulated differently from those in the myometrium and that the control of sensitivity to relaxin, as expressed by receptor concentrations, differs in the two tissues. Events occurring after relaxin–receptor interaction are not well understood; cAMP levels increase in the cervices of rats (Cheah and Sherwood, 1980) and pigs (Judson *et al.*, 1980). The consequence of this action is, as yet, unknown.

5.1.3. Prostaglandins

Prostaglandins have been shown to be effective in cervical ripening when administered orally (Pearce, 1977; Valentine, 1977), extraamniotically (Calder *et al.*, 1977), or intravaginally (MacKenzie and Embrey, 1977). Locally administered forms of PGE_2, including intracervical gels and vaginal suppositories, have been found to be effective and safe methods for preinduction of cervical ripening as well as for induction of labor (Ekman *et al.*, 1983).

Evidence suggests that PGE_2 has a local ripening effect on the gravid cervix independent of uterine contractile activity when administered in a fashion that avoided exposure of the extraamniotic space to PGE_2 gel. Intracervical PGE_2 in the first-trimester pregnant woman caused ultrastructural changes characteristic of cervical ripening at term (Theobald *et al.*, 1982). Intracervical administration of PGE_2 in gel to women for cervical priming and induction of labor significantly increased collagenolytic activity in cervical biopsy specimens (Ekman *et al.*, 1986). Others, however, have suggested that enzymatic degradation of collagen does not play a predominent role in prostaglandin-induced cervical ripening (Rath *et al.*, 1987). Instead, changes in glycosaminoglycan content may be more important.

The administration of either PGE_2 or $PGF_{2\alpha}$ to rats on day 18 of pregnancy doubled the extensibility of the cervix by day 19 (Hollingsworth *et al.*, 1980). The administration of progesterone had no effect alone or on the effect of PGE_2 but inhibited the action of $PGF_{2\alpha}$. The effects of $PGF_{2\alpha}$, but not those of PGE_2, also were inhibited by ovariectomy in the rat. These results suggest that PGE_2 and $PGF_{2\alpha}$ act differently in promoting cervical extensibility in the rat. Whereas PGE_2 might act directly on the cervix, the effects of $PGF_{2\alpha}$ appear to be mediated by luteal regression. Prostaglandin $F_{2\alpha}$ infusion into pigs during late pregnancy caused a relaxin surge from the ovaries into the circulation (Sherwood *et al.*, 1969). The administration of indomethacin delayed the release of relaxin before parturition. These findings, along with those of Hollingsworth *et al.*, (1980), suggest that effects of prostaglandins on the cervix might be mediated by relaxin. However, induction of labor in women with $PGF_{2\alpha}$ did not cause a significant elevation in serum relaxin immunoactivity (Hochman *et al.*, 1978), implying that $PGF_{2\alpha}$ acts directly on the cervix. It is possible that the effects of relaxin are mediated by prostaglandins, but definitive experiments to test this relationship have not been carried out.

5.2. Relationship between Cervical Maturation and Myometrial Contractions

Cervical maturation and myometrial contractions can occur independently. Yet, the two processes occur together temporally and appear to be regulated similarly. In patients with prolonged pregnancies, those with unripe cervices were only rarely good candidates for induction of labor (Harris *et al.*, 1983). In a retrospective study by Lange *et al.* (1982), about 1200 patients were analyzed for a correlation between cervical status and inducibility of labor. Cervical dilatation and effacement were the best predictors of the onset of labor. Similar conclusions have been drawn by other investigators (Bouyer *et al.*, 1986).

Some of the similarities in regulation of cervical changes and myometrial activity have been pointed out by Huszar *et al.* (1986). They include the following.

1. Progesterone dominance during pregnancy is associated with a firmly closed cervix and a quiescent myometrium.
2. At term, estrogen or rising estrogen/progesterone ratios have been shown to be important in increased uterine contractility and in cervical ripening.
3. Prostaglandin $F_{2\alpha}$ and other prostanoids have direct stimulatory effects on uterine contractility as well as direct and indirect actions on cervical ripening. Whereas prostaglandins at low levels bring about cervical maturation, administration of PGE_2 and $PGF_{2\alpha}$ in larger amounts will induce myometrial contractions in pregnant women at any stage of gestation.

On the basis of the preceding, the following sequence of events in initiation of labor in humans is proposed. The concentration of oxytocin receptors increases during gestation and doubles before the onset of labor. There is more than a 100-fold increase in the concentration of myometrial oxytocin receptors from the beginning to the end of gestation. This increase is probably caused by increasing estrogen concentrations in the blood, but additional factors may be involved. Although there is little or no change in circulating oxytocin levels, increased receptor levels allow more oxytocin to be bound to myometrial cells. The threshold to oxytocin is then lowered to the point at which the smooth muscle cells contract in response to basal or slightly elevated levels of oxytocin.

Oxytocin also binds to decidual cell receptors, which are up-regulated during pregnancy, stimulating prostaglandin synthesis. Prostaglandins likely enhance the sensitivity of the myometrial response to oxytocin, possibly at the postreceptor level. Part or all of the effects of increased prostaglandin levels may be to increase the number of gap junctions between myometrial cells (Garfield *et al.*, 1980). Cells that do not have oxytocin receptors or cells with unoccupied receptors may be coupled chemically to those that do. As a result, a given concentration of oxytocin elicits a magnified response, which could appear as an increase in sensitivity to oxytocin.

During the expulsive stage of labor, oxytocin levels in the blood increase, and a greater fraction of oxytocin receptor sites is occupied. Myometrial contractions are enhanced, and delivery is facilitated. Following parturition, the concentration of uterine oxytocin receptors falls off rapidly, dampening the response of the uterus to elevated levels of oxytocin in the blood during lactation.

Progesterone and estrogens play a facilitatory role in the initiation of labor. As suggested by Fuchs and Fuchs (1984), oxytocin may be important for the initial phase of labor, whereas increased synthesis of $PGF_{2\alpha}$ would be essential for the progression of labor. Prostaglandin E_2 may play a role in the ripening of the cervix as an essential step for successful parturition. Cervical dilitation appears to operate independently of uterine contractions, but both processes may be governed by some of the same operators. Relaxin serves to prepare the cervix for delivery.

7. *Unification of Mechanisms Proposed for Spontaneous Labor Induction*

Although different mechanisms of labor initiation operate in different species, physiological processes generally tend to be more similar than different in related species. Accordingly, a unified mechanism for initiation of labor should take into account the various models that have been proposed. The postulate that labor is initiated by increased sensitivity of the myometrium to oxytocin and that this is accomplished by up-regulation of oxytocin receptors is compatible with other hypotheses because of the following observations.

7.1. *Prostaglandins*

Demonstration of elevated oxytocin receptors in decidual tissues at the end of pregnancy makes it possible to reconcile hypotheses that have excluded either prostaglandins or oxytocin as natural labor initiators. Elevation of prostaglandin levels during labor may be the result of oxytocin stimulation of decidual cells. The role for prostaglandins, whether uterotonic, supportive of the uterotonic effects of oxytocin, or acting instead at other loci, remains to be

clarified. Oxytocin, therefore, may be capable of serving a dual function in initiating labor by its effects on both myometrium and endometrium. Whether oxytocin receptors on fetal membranes are capable of stimulating prostaglandin release remains to be clarified.

In species like the rat that depend on the luteal synthesis of progesterone for maintenance of pregnancy, administration of a single dose of $PGF_{2\alpha}$ beyond 15 days of pregnancy results in premature termination of gestation with a corresponding increase in oxytocin receptor concentrations in the myometrium. Because this action of $PGF_{2\alpha}$ can be prevented by simultaneous administration of progesterone, it is likely that the increase in oxytocin receptor concentration is the result of the luteolytic activity of PGF. In species like the human that do not require an intact corpus luteum for maintenance of pregnancy, prostaglandins may induce increases in oxytocin receptor concentration by other mechanisms that remain to be studied or by sensitizing the myometrium to basal levels of oxytocin in the blood.

7.2. *Estrogen/Progesterone*

Changes in uterine oxytocin receptor concentrations are regulated by estrogen/progesterone concentrations. Estrogen domination of the uterus, which may result from increases in the ratio of estrogen/progesterone concentrations in blood or from increases in estrogen levels alone, is compatible with the progesterone block theory of Csapo (1956). An absence of progesterone withdrawal in species such as the human and guinea pig does not necessarily preclude estrogen stimulation of oxytocin receptor concentrations in the myometrium and possibly the endometrium during pregnancy, particularly the latter stages.

7.3. *Fetal ACTH*

In the sheep, there is strong evidence that implicates the fetal release of ACTH in initiation of labor. Resultant elevations in glucocorticoids, in turn, likely lower progesterone and increase estrogen levels in the maternal circulation. These changes result in an increase in concentrations of oxytocin receptors. The oxytocin receptor mechanism is compatible with other models that have been suggested for the initiation of labor. The fetus may be involved in coordinating activities leading to labor through its influence on placental production of estrogen and possibly its secretion of neurohypophyseal hormones and other stimulators (or inhibitors) of prostaglandin synthesis. In conclusion, the up- and down-regulation of receptors illustrate that it is important to understand that uterine status cannot necessarily be assessed from circulating hormone levels alone. An understanding of factors involved in initiating labor therefore must take into consideration levels of receptor as well as those of circulating hormones.

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8. *References*

Abel, M. H., and Baird, D. T., 1980, The effect of 17β-estradiol and progesterone on prostaglandin production by human endometrium maintained in organ culture. *Endocrinology* **106**:1599–1606.

Abel, M. H., and Kelly, R. W., 1979, Differential production of prostaglandins within the human uterus, *Prostaglandins* **18**:821–828.

Abel, M. H., Smith, S. K., and Baird, D. T., 1980, Suppression of concentration of endometrial prostaglandin in early intra-uterine and ectopic pregnancy in women, *J. Endocrinol.* **85**:379–386.

Adelstein, R. S., Conti, M. A., Hathaway, D. R., and Klee, C. B., 1978, Phosphorylation of smooth muscle myosin light chain kinase by the catalytic subunit of adenosine 3′:5′-monophosphate-dependent protein kinase. *J. Biol. Chem.* **253**:8347–8350.

Ahlquist, R. P., 1966, The adrenergic receptor, *J. Pharm. Sci.* **55**:359–367.

Aiken, J. W., 1972, Aspirin and indomethacin prolong parturition in rats. Evidence that prostaglandins contribute to expulsion of foetus, *Nature* **240**:21–25.

Åkerlund, M., Strömberg, P., Hauksson, A., Andersen, L. F., Lyndrup, J., Trojnar, J., and Melin, P., 1987, Inhibition of uterine contractions of premature labour with an oxytocin analogue, *Br. J. Obstet. Gynaecol.* **94**:1040–1044.

Åkerman, K. E. O., and Wikström, M. K. F., 1979, (Ca^{2+} + Mg^{2+})-stimulated ATPase activity of rabbit myometrium plasma membrane is blocked by oxytocin *FEBS Lett.* **97**:283–287.

Alexandrova, M., and Soloff, M. S., 1980a, Oxytocin receptors and parturition. I. Control of oxytocin receptor concentration in the rat myometrium at term, *Endocrinology* **106**:730–735.

Alexandrova, M., and Soloff, M. S., 1980b, Oxytocin receptors and parturition. III. Increases in estrogen receptor and oxytocin receptor concentrations in the rat myometrium during $PGF_{2\alpha}$-induced abortion, *Endocrinology* **106**:739–743.

Alexandrova, M., and Soloff, M. S., 1980c, Oxytocin receptors and parturition in the guinea pig, *Biol. Reprod.* **22**:1106–1111.

Anderson, L. L., Bowerman, A. M., and Melampy, R. M., 1965, Oxytocin on ovarian function in cycling and hysterectomized heifers, *J. Anim. Sci.* **24**:964–968.

Andersson, R. G. G., Berg, G., Johansson, S.R. M., and Rydén, G., 1980, Effects of non-selective and selective beta-adrenergic agonists on spontaneous contractions and cyclic AMP levels in myometrial strips from pregnant women, *Gynecol. Obstet. Invest.* **11**:286–293.

Armstrong, D. T., and Hansel, W., 1959, Alteration of the bovine estrous cycle with oxytocin, *J. Dairy Sci.* **42**:533–542.

Bauknecht, T., Krahe, B., Rechenbach, U., Zahradnik, H. P., and Breckwoldt, M., 1981, Distribution of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ receptors in human myometrium, *Acta Endocrinol. (Kbh.)* **98**:446–450.

Bejar, R., Curbelo, V., Davis, C., and Gluck, L., 1981, Premature labor. II. Bacterial sources of phospholipase, *Obstet. Gynecol.* **57**:479–482.

Bell, C., and Malcolm, S. J., 1978, Observations on the loss of catecholamine fluorescence from intrauterine adrenergic nerves during pregnancy in the guinea pig, *J. Reprod. Fertil.* **53**:51–58.

Bercu, B. B., Hyashi, A., Poth, M., Alexandrova, M., Soloff, M. S., and Donahoe, P. K., 1980, LHRH-induced delay of parturition, *Endocrinology* **107**:504–508.

Billah, M. M., and Johnston, J. M., 1983, Identification of phospholipid platelet-activating factor (1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) in human amniotic fluid and urine, *Biochem. Biophys. Res. Commun.* **113**:51–58.

Billah, M. M., Di Renzo, G. C., Ban, C., Truong, C. T., Hoffman, D. R., Anceschi, M. M., Bleasdale, J. E., and Johnston, J. M., 1985, Platelet-activating factor metabolism in human amnion and the responses of this tissue to extracellular platelet-activating factor, *Prostaglandins* **30**:841–850.

Blatchley, F. R., Donovan, B. T., Poyser, N. L., Horton, E. W., Thompson, C. J., and Los, M., 1971, Identification of prostaglandin $F_{2\alpha}$ in the utero-ovarian blood of guinea-pig after treatment with estrogen, *Nature* **230**:243–244.

Bleasdale, J. E., Okazaki, T., Sagawa, N., Di Renzo, G. C., Okita, J. R., MacDonald, P. C., and Johnston, J. M., 1983, The mobilization of arachidonic acid for prostaglandin production during parturition, in: *Initiation of Parturition: Prevention of Prematurity* (P. C. MacDonald and J. Porter, eds.), Ross Laboratories, Columbus, OH, pp. 129–137.

Bothwell, W., Verburg, M., Wynalda, M., Daniels, E. G., and Fitzpatrick, F. A., 1982, A radioimmunoassay for the unstable pulmonary metabolite of prostaglandin E_1 and E_2: An indirect index of their *in vivo* disposition and pharmacokinetics, *J. Pharmacol. Exp. Ther.* **220**:229–235.

Bouyer, J., Papiernik, E., Dreyfus, J., Collin, D., Winisdoerffer, B., and Gueguen, S., 1986, Maturation signs of the cervix and prediction of preterm birth, *Obstet. Gynecol.* **68**:209–214.

Boyle, M. B., MacLusky, N. J., Naftolin, F., and Kaczmarek, L., 1987, Hormonal regulation of potassium channel mRNA in cycling and pregnant myometrium, *Nature* **330**:373–375.

Brennecke, S. P., Lenton, E. A., Turnbull, A. C., and Mitchell, M. D., 1982, Inhibition of prostaglandin synthase by maternal plasma factor(s) in early human pregnancy, *Br. J. Obstet. Gynaecol.* **89**:612–616.

Brennecke, S. P., Humphreys, J., Bryce, R. L., Teasdale, W. P., Turnbull, A. C., and Mitchell, M. D., 1984, Maternal plasma inhibition of prostaglandin synthase during human pregnancy, parturition and the puerperium, *Br. J. Obstet. Gynaecol.* **91**:349–352.

Brennecke, [illegible], Castle, B. M., Demers, L. M., and Turnbull, A. [illegible], 1985, Maternal plasma prostaglandin E_2 metabolite levels during human pregnancy and parturition, *Br. J. Obstet. Gynaecol.* **92**:345–349.

Brummer, H. C., 1971, Interaction of E prostaglandins and syntocinon on the pregnant human myometrium, *J. Obstet. Gynaecol. Br. Commonw.* **78**:305–309.

Bryant-Greenwood, G. D., 1982, Relaxin as new hormone, *Endocrine Rev.* **3**:62–90.

Cabrol, D., Bouviér d'Yvoire, M., Mermet, E., Cedard, L., Sureau, C., and Baulieu, E. E., 1985, Induction of labour with mifepristone after intrauterine fetal death, *Lancet* **2**:1019.

Calder, A. A., Embrey, M. P., and Tait, T., 1977, Ripening of the cervix with extra-amniotic prostaglandin E_2 in viscous gel before induction of labor, *Br. J. Obstet. Gynaecol.* **84**:264–268.

Caldeyro-Barcia, R., and Sereno, J., 1961, The response of the human uterus to oxytocin throughout pregnancy, in: *Oxytocin* (R. Caldeyro-Barcia and H. Heller, eds.), Pergamon Press, Oxford, pp. 177–202.

Caldwell, B. V., Tillson, S., Brock, W. A., and Speroff, L., 1972, The effects of exogenous progesterone and estradiol on prostaglandin F levels in ovariectomized ewes, *Prostaglandins* **1**:217–238.

Carsten, M. E., and Miller, J. D., 1985, Ca^{2+} release by inositol trisphosphate from Ca^{2+}-transporting microsomes derived from uterine sarcoplasmic reticulum, *Biochem. Biophys. Res. Commun.* **130**:1027–1031.

Casey, M. L., and MacDonald, P. C., 1984, Endocrinology of preterm birth, *Clin. Obstet. Gynecol.* **27**:562–571.

Casey, M. L., Cutrer, S. I., and Mitchell, M. D., 1983a, Origin of prostanoids in human amniotic fluid: The fetal kidney as a source of amniotic fluid prostanoids, *Am. J. Obstet. Gynecol.* **147**:547–551.

Casey, M. L., MacDonald, P. C., and Mitchell, M. D., 1983b, Stimulation of prostaglandin E_2 production in amnion cells in culture by a substance(s) in human fetal and adult urine, *Biochem. Biophys. Res. Commun.* **114**:1056–1063.

Casey, M. L., MacDonald, P. C., and Mitchell, M. D., 1984, Characterization of prostaglandin formation by human amnion cells in monolayer culture, *Prostaglandins* **27**:421–427.

Challis, J. R. G., and Vaughan, M., 1987, Steroid synthetic and prostaglandin metabolizing activity is present in different cell populations from human fetal membranes and decidua, *Am. J. Obstet. Gynecol.* **157**:1474–1481.

Chamley, W. A., Bagoyo, M. M., and Bryant-Greenwood, G. D., 1977, *In vitro* response of relaxin-treated rat uterus to prostaglandins and oxytocin, *Prostaglandins* **14**:763–769.

Chan, W. Y., 1977, Relationship between the uterotonic action of oxytocin and prostaglandins: Oxytocin action and release of PG-activity in isolated nonpregnant and pregnant rat uteri, *Biol. Reprod.* **17**:541–548.

Chan, W. Y., 1980, The separate uterotonic and prostaglandin-releasing actions of oxytocin. Evidence and comparison with angiotensin and methacholine in the isolated rat uterus, *J. Pharmacol. Exp. Ther.* **213**:575–579.

Chan, W. Y., 1987, Enhanced prostaglandin synthesis in the parturient rat uterus and its effects on myometrial oxytocin receptor concentrations, *Prostaglandins* **34**:889–902.

Chang, W.-C., Nakao, J., Murota, S.-I., and Tai, H.-H., 1983, Induction of fatty acid cyclooxygenase in rat aortic smooth muscle cells by estradiol, *Prostaglandins Leukotrienes Med.* **10**:33–37.

Cheah, S. H., and Sherwood, O. D., 1980, Target tissues for relaxin in the rat: Tissue distribution of injected ^{125}I-labeled relaxin and tissue changes in adenosine 3′,5′-monophosphate levels after *in vitro* relaxin incubation, *Endocrinology* **106**:1203–1209.

Chegini, N., and Rao, C. V., 1988, The presence of leukotriene C_4- and prostacyclin-binding sites in nonpregnant human uterine tissue, *J. Clin. Endocrinol. Metab.* **66**:76–87.

Chegini, N., Rao, C. V., Wakim, N., and Sanfilippo, J., 1986, Prostaglandin binding to different cell types of human uterus: Quantitative light microscope autoradiographic study, *Prostaglandins Leukotrienes Med.* **22**:129–138.

Chester, R., Dukes, M., Slater, S. R., and Walpole, A. L., 1972, Delay of parturition in the rat by anti-inflammatory agents which inhibit the biosynthesis of prostaglandins, *Nature* **240**:37–38.

Chew, C. S., and Rinard, G. A., 1979, Glycogen levels in the rat myometrium at the end of pregnancy and immediately postpartum, *Biol. Reprod.* **20**:1111–1114.

Clark, J. H., and Markaverich, B. M., 1984, The agonistic and antagonistic actions of estriol, *J. Steroid Biochem.* **20**:1005–1013.

Clark, J. H., Hsueh, A. J. W., and Peck, E. J., Jr., 1977, Regulation of estrogen receptor replenishment by progesterone, *Ann. N.Y. Acad. Sci.* **286**:161–179.

Clegg, P. C., Hall, W. J., and Pickles, V. R., 1966, The action of ketonic prostaglandins on the guinea pig myometrium, *J. Physiol. (Lond.)* **183**:123–146.

Cooke, R. G., and Homeida, A. M., 1985, Suppression of prostaglandin $F_{2\alpha}$ release and delay of luteolysis after active immunization against oxytocin in the goat, *J. Reprod. Fertil.* **75**:63–68.

Cornette, J. C., Harrison, K. L., and Ki[illegible], K. T., 1974, Measurement of prostaglandin $F_{2\alpha}$ metabolites by radioimmunoassay, *Prostaglandins* **5**:155–164.

Crankshaw, D. J., 1987, The sensitivity of the longitudinal and circular muscle layers of the rat's myometrium to oxytocin *in vitro* during pregnancy, *Can. J. Physiol. Pharmacol.* **65**:773–777.

Crankshaw, D. J., Crankshaw, J., Branda, L. A., and Daniel, E. E., 1979, Receptors for E type prostaglandins in the plasma membrane of nonpregnant human myometrium, *Arch. Biochem. Biophys.* **198**:70–77.

Creange, J. E., Schane, H. P., Anzalone, A. J., and Potts, G. O., 1978, Interruption of pregnancy in rats by azastene, an inhibitor of ovarian and adrenal steroidogenesis, *Fertil. Steril.* **30**:86–90.

Csapo, A. I., 1956, Progesterone block, *Am. J. Anat.* **98**:273–291.

Csapo, A. I., 1975, The "seesaw" theory of the regulatory mechanism of pregnancy, *Am. J. Obstet. Gynecol.* **121**:578–581.

Csapo, A. I., and Resch, B. A., 1979, Induction of preterm labor in the rat by antiprogesterone, *Am. J. Obstet. Gynecol.* **134**:823–827.

Csapo, A. I., and Takeda, H., 1965, Effect of progesterone on the electrical activity and intra-uterine pressure of pregnant and parturient rabbits, *Am. J. Obstet. Gynecol.* **91**:221–231.

Csapo, A. I., and Wiest, W. G., 1969, An examination of the quantitative relationship between progesterone and maintenance of pregnancy, *Endocrinology* **85**:735–746.

Csapo, A. I., Puri, C. P., Tarro, S., and Henzl, M. R., 1982, Deactivation of the uterus during normal and premature labor by the calcium antagonist nicardipine, *Am. J. Obstet. Gynecol.* **142**:483–491.

Danforth, D. N., 1947, Fibrous nature of human cervix, and its relation to isthmic segment in gravid and nongravid uteri, *Am. J. Obstet. Gynecol.* **53**:541–560.

Danforth, D. N., Veis, A., Breen, M., Weinstein, H. G., Buckingham, J. C., and Manalo, P., 1974, The effect of pregnancy and labor on the human cervix: Changes in collagen, glycoproteins, and glycosaminoglycans, *Am. J. Obstet. Gynecol.* **120**:641–651.

Darne, J., McGarrigle, H. H. G., and Lachelin, G. C. L., 1987, Saliva oestriol, oestradiol, oestrone and progesterone levels in pregnancy: Spontaneous labour at term is preceded by a rise in the saliva oestriol:progesterone ratio, *Br. J. Obstet. Gynaecol.* **94**:227–235.

Dey, F. L., Fisher, C., and Ranson, S. W., 1941, Disturbances in pregnancy and labor in guinea pigs with hypothalamic lesions, *Am. J. Obstet. Gynecol.* **42**:459–466.

Diczfalusy, E., 1974, Endocrine functions of the human fetus and placenta, *Am. J. Obstet. Gynecol.* **119**:419–433.

Di Renzo, G. C., Johnston, J. M., Okazaki, T., Okita, J. R., MacDonald, P. C., and Bleasdale, J. E., 1981, Phosphatidylinositol-specific phospholipase C in fetal membranes and uterine decidua, *J. Clin. Invest.* **67**:847–856.

Di Renzo, G. C., Anceschi, M. M., and Bleasdale, J. E., 1984a, Beta-adrenergic stimulation of prostaglandin production by human amnion tissue, *Prostaglandins* **27**:37–49.

Di Renzo, G. C., Venincasa, M. D., and Bleasdale, J. E., 1984b, The identification and characterization of β-adrenergic receptors in human amnion tissue, *Am. J. Obstet. Gynecol.* **148**:398–405.

Divers, W. A., Jr., Wilkes, M. M., Babaknia, A., and Yen, S. S. C., 1981, An increase in catecholamines and metabolites in the amniotic fluid compartment from middle to late gestation, *Am. J. Obstet. Gynecol.* **139**:483–486.

Downing, S. J., and Sherwood, O. D., 1985a, The physiological role of relaxin in the pregnant rat. I. The influence of relaxin on parturition, *Endocrinology* **116**:1200–1205.

Downing, S. J., and Sherwood, O. D., 1985b, The physiological role of relaxin in the pregnant rat. II. The influence of relaxin on uterine contractile activity, *Endocrinology* **116**:1206–1214.

Downing, S. J., and Sherwood, O. D., 1985c, The physiological role of relaxin in the pregnant rat. III. The influence of relaxin on cervical extensibility, *Endocrinology* **116**:1215–1220.

Downing, S. J., Bradshaw, J. M. C., and Porter, D. G., 1980, Relaxin improves the coordination of rat myometrial activity *in vivo*, *Biol. Reprod.* **23**:899–903.

Dubin, N. H., Ghodgaonkar, R. B., and King, T. M., 1979, Role of prostaglandin production in spontaneous and oxytocin-induced uterine contractile activity in *in vitro* pregnant rat uteri, *Endocrinology* **105**:47–51.

Edman, K. A. P., and Schild, H. O., 1962, The need for the calcium in the contractile responses induced by acetylcholine and potassium in the rat uterus, *J. Physiol. (Lond.)* **161**:424–441.

Ekman, G., Forman, A., Marsál, K., and Ulmsten, U., 1983, Intravaginal versus intracervical application of prostaglandin E_2 in viscous gel for cervical priming and induction of labor at term in patients with an unfavorable cervical state, *Am. J. Obstet. Gynecol.* **147**:657–661.

Ekman, G., Malmström, A., Uldbjerg, N., and Ulmsten, U., 1986, Cervical collagen: An important regulator of cervical function in term labor, *Obstet. Gynecol.* **67**:633–636.

Ellendo F., Forsling, M., Parvizi, N., Williams, H., Tave M., and Smidt, D., 1979, Plasma oxytocin and vasopressin concentrations in response to prostaglandin injection into the pig, *J. Reprod. Fertil.* **56**:573–577.

Embrey, M. P., 1969, The effect of prostaglandins on human pregnant uterus, *J. Obstet. Gynaecol. Br. Commonw.* **76**:783–789.

Falck Larsen, J., Kern Hansen, M., Hesseldahl, H., Kristoffersen, K., Larsen, P. K., Osler, M., Weber, J., Eldon, K., and Lange, A., 1980, Ritodrine in the treatment of preterm labour. A clinical trial to compare a standard treatment with three regimens involving the use of ritodrine, *Br. J. Obstet. Gynaecol.* **87**:949–957.

Falck Larsen, J., Eldon, K., Lange, A. P., Leegaard, M., Osler, M. Sederberg Olsen, J., and Permin, M., 1986, Ritodrine in the treatment of preterm labor: Second Danish multicenter study, *Obstet. Gynecol.* **67**:607–613.

Ferguson, J. K. W., 1941, A study of the motility of the intact uterus at term, *Surg. Gynecol. Obstet.* **73**:359–366.

Fields, P. A., and Larkin, L. H., 1980, Enhancement of uterine cervix extensibility in oestrogen-primed mice following administration of relaxin, *J. Endocrinol.* **87**:147–152.

Fisher, C., Magoun, H. W., and Ranson, S. W., 1938, Dystocia in diabetes insipidus. The relation of pituitary oxytocin to parturition, *Am. J. Obstet. Gynecol.* **36**:1–9.

Fitzpatrick, F. A., Aguirre, R., Pike, J. E., and Lincoln, F. H., 1980, The stability of 13,14-dihydro-15 keto-PGE_2, *Prostaglandins* **19**:917–931.

Flint, A. P. F., and Sheldrick, E. L., 1982, Ovarian secretion of oxytocin is stimulated by prostaglandins, *Nature* **297**:587–588.

Flint, A. P. F., Leat, W. M. F., Sheldrick, E. L., and Stewart, H. J., 1986, Stimulation of phosphoinositide hydrolysis by oxytocin and the mechanism by which oxytocin controls prostaglandin synthesis in the ovine endometrium, *Biochem. J.* **237**:797–805.

Flower, R. J., and Blackwell, G. J., 1979, Anti-inflammatory steroids induce biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation, *Nature* **278**:456–459.

Forman, A., Gandrup, P., Andersson, K.-E., and Ulmsten, U., 1982, Effects of nifedipine on oxytocin- and prostaglandin $F_{2\alpha}$-induced activity in the postpartum uterus, *Am. J. Obstet. Gynecol.* **144**:665–670.

Fox, A. W., Friedman, P. A., and Abel, P. W., 1987, Vasopressin receptor mediated contraction and [^{3}H]inositol metabolism in rat tail artery, *Eur. J. Pharmacol.* **135**:1–10.

Fuchs, A.-R., 1972, Prostaglandin effects on rat pregnancy. I. Failure of induction of labor, *Fertil. Steril.* **23**:410–416.

Fuchs, A.-R., 1985, Oxytocin in animal parturition, in: *Oxytocin. Clinical and Laboratory Studies* (J. A. Amico, and A. G. Robinson, eds.), Elsevier, Amsterdam, pp. 207–235.

Fuchs, A.-R., and Fuchs, F., 1984, Endocrinology of human parturition: A review, *Br. J. Obstet. Gynaecol.* **91**:948–967.

Fuchs, A.-R., and Poblete, V. R., Jr., 1970, Oxytocin and uterine function in pregnant and parturient rats, *Biol. Reprod* **2**:387–400.

Fuchs, A.-R., Mok, E., and Sundaram, K., 1974, Luteolytic effects of prostaglandins in rat pregnancy, and reversal by luteinizing hormone, *Acta Endocrinol. (Kbh).* **76**:583–596.

Fuchs, A.-R., Fuchs, F., Husslein, P., Soloff, M. S., and Fernstrom, M., 1982, Oxytocin receptors and human parturition: A dual role for oxytocin in the initiation of labor, *Science* **215**:1396–1398.

Fuchs, A.-R., Periyasamy, S., Alexandrova, M., and Soloff, M. S., 1983a, Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium. Effects of ovarian steroids, *Endocrinology* **113**:742–749.

Fuchs, A.-R., Goeschen, K., Husslein, P., Rasmussen, A. B., and Fuchs, F., 1983b, Oxytocin and the initiation of human parturition. III. Plasma concentrations of oxytocin and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ in spontaneous and oxytocin-induced labor at term, *Am. J. Obstet. Gynecol.* **147**:497–502.

Fuchs, A.-R., Fuchs, F., Husslein, P., and Soloff, M. S., 1984, Oxytocin receptors in human uterus during pregnancy and parturition, *Am. J. Obstet. Gynecol.* **150**:734–741.

Fuchs, A.-R., Fuchs, F., and Soloff, M. S., 1985, Oxytocin receptors in nonpregnant human uterus, *J. Clin. Endocrinol. Metab.* **60**:37–41.

Fukai, H., Den, K., Sakamoto, H., Kodaira, H., Uchida, F., and Takagi, S., 1984, Study of oxytocin receptor: II. Oxytocin and prostaglandin $F_{2\alpha}$ receptors in human myometria and amnion–decidua complex during pregnancy and labor, *Endocrinol. Jpn.* **31**:565–570.

Furr, B. J. A., Valcaccia, B., and Challis, J. R. G., 1976, The effects of Nolvadex (tamoxifen citrate; ICI 46,474) on pregnancy in rabbits, *J. Reprod. Fertil.* **48**:367–369.

Garfield, R. E., Kannan, M. S., and Daniel, E. E., 1980, Gap junction formation in myometrium: Control by estrogens, progesterone, and prostaglandins, *Am. J. Physiol.* **238**:C81–C89.

Gennser, G., Ohrlander, S., and Eneroth, P., 1977, Fetal cortisol and the initiation of labour in the human, in: *The Fetus and Birth, Ciba Foundation Symposium*, Elsevier/Excerpta Medica/North-Holland, Amsterdam, pp. 401–426.

Gerzer, R., Brash, A. R., and Hardman, J. G., 1986, Activation of soluble guanylate cyclase by arachidonic acid and 15-lipoxygenase products, *Biochim. Biophys. Acta* **886**:383–389.

Giannopoulos, G., Goldberg, P., Shea, T. B., and Tulchinsky, D., 1980, Unoccupied and occupied estrogen receptors in myometrial cytosol and nuclei from nonpregnant and pregnant women, *J. Clin. Endocrinol. Metab.* **51**:702–710.

Gibb, W., Lavoie, J.-C., and Roux, J. F., 1978, 3β-Hydroxysteroid dehydrogenase activity in human fetal membranes, *Steroids* **32**:365–372.

Gietzen, K., Seiler, S., Fleischer, S., and Wolf, H. U., 1980, Reconstitution of the Ca^{2+}-transport system of human erythrocytes, *Biochem. J.* **188**:47–54.

Gillespie, A., 1972, Prostaglandin–oxytocin enhancement and potentiation and their clinical applications, *Br. Med. J.* **1**:150–152.

Gillespie, A., Brummer, H. C., and Chard, T., 1972, Oxytocin release by infused prostaglandin, *Br. Med. J.* **1**:543–544.

Glasser, S. R., Northcutt, R. C., Chytil, F., and Strott, C. A., 1972, The influence of an antisteroidogenic drug (aminoglutethimide phosphate) on pregnancy maintenance, *Endocrinology*, **90**:1363–1370.

Golichowski, A. M., 1986, Biochemical basis of cervical maturation, in: *The Physiology and Biochemistry of the Uterus in Pregnancy and Labor* (G. Huszar, ed.), CRC Press, Boca Raton, FL, pp. 261–280.

Golichowski, A. M., and Tzeng, D. Y., 1985, Binding of the calcium antagonist [^{3}H]nitrendipine to human myometrial plasmalemma, *Biol. Reprod.* **33**:1105–1112.

Gordon, A. J., and Calder, A. A., 1977, Oestradiol applied locally to ripen the unfavourable cervix, *Lancet* **2**:1319–1321.

Goren, H. J., Geonzon, R. M., Hollenberg, M. D., Lederis, K., and Morgan, D. O., 1980, Oxytocin action: Lack of correlation between receptor number and tissue responsiveness, *J. Supramol. Struct.* **14**:129–138.

Granström, E., Hamberg, M., Hansson, G., and Kindahl, H., 1980, Chemical instability of 15-keto-13,14-dihydro-PGE_2: The reason for low assay reliability, *Prostaglandins* **19**:933–957.

Gréen, K., Bygdeman, M., Toppozoda, M., and Wiqvist, N., 1974, The role of $PGF_{2\alpha}$ in human parturition. Endogenous plasma levels of 15-keto-13,14-dihydroprostaglandin $F_{2\alpha}$ during labor, *Am. J. Obstet. Gynecol.* **120**:25–31.

Grover, A. K., Kwan, C.-Y., Luchowski, E., Daniel, E. E., and Triggle, D. J., 1984, Subcellular distribution of [^{3}H]nitrendipine binding in smooth muscle, *J. Biol. Chem.* **259**:2223–2226.

Gutknecht, G. D., Duncan, G. W., and Wyngarden, L. J., 1972, Inhibition of prostaglandin $F_{2\alpha}$ or LH induced luteolysis in the pseudopregnant rabbit by 17β-estradiol, *Proc. Soc. Exp. Biol. Med.* **130**:406–413.

Habenicht, A. J. R., Goerig, M., Grulich, J., Rothe, D., Gronwald, R., Loth, U., Schettler, G., Kommerell, B., and Ross, R., 1985, Human platelet-derived growth factor stimulates prostaglandin synthesis by activation and by rapid *de novo* synthesis of cyclooxygenase, *J. Clin. Invest.* **75**:1381–1387.

Haldar, J., Kupfer, L., and Sokol, H. W., 1982, Decreased sensitivity to oxytocin of uteri from homozygous Brattleboro rats, *Ann. N.Y. Acad. Sci.* **394**:46–49.

Harden, T. K., 1983, Agonist-induced desensitization of the β-adrenergic receptor-linked adenylate cyclase, *Pharmacol. Rev.* **35**:5–32.

Harris, B. A., Jr., Huddleston, J. F., Sutliff, G., and Perlis, H. W., 1983, The unfavorable cervix in prolonged pregnancy, *Obstet. Gynecol.* **62**:171–174.

Ham, E. A., Cirillo, V. J., Zanetti, M. E., and Kuehl, F. A., Jr., 1975, Estrogen-directed synthesis of specific prostaglandins in uterus, *Proc. Natl. Acad. Sci. U.S.A.* **72**:1420–1427.

Hertelendy, F., 1973, Block of oxytocin-induced parturition and oviposition by prostaglandin inhibitors, *Life Sci.* **13**:1581–1589.

Hilliard, J., 1973, Corpus luteum function in guinea pigs, hamsters, rats, mice and rabbits, *Biol. Reprod.* **8**:203–221.

Hirata, F., Matsuda, K., Notsu, Y., Hattori, T., and del Carmine, R., 1984, Phosphorylation at a tyrosine residue of lipomodulin in mitogen-stimulated murine thymocytes, *Proc. Natl. Acad. Sci. U.S.A.* **81**:4717–4721.

Hochman, J., Weiss, G., Steinetz, B. G., and O'Byrne, E. M., 1978, Serum relaxin concentrations in prostaglandin- and oxytocin-induced labor in women, *Am. J. Obstet. Gynecol.* **130**:473–474.

Hoffman, B. B., Lavin, T. N., Lefkowitz, R. J., and Ruffolo, R. R., Jr., 1981, Alpha adrenergic receptor subtypes in rabbit uterus: Mediation of myometrial contractions and regulation by estrogens, *J. Pharmacol. Exp. Ther.* **219**:290–295.

Hofmann, G. E., Rao, C. V., Barrows, G. H., and Sanfilippo, J. S., 1983, Topography of human uterine

pr[illegible]landin E and $F_{2\alpha}$ receptors and their profiles durin[illegible] [illegible]iological states, *J. Clin. Endocrinol. Metab.* **56**:360–366.

Hollingsworth, M., Gallimore, S., and Isherwood, C. N. M., 1980, Effects of prostaglandins $F_{2\alpha}$ and E_2 on cervical extensibility in the late pregnant rat, *J. Reprod. Fertil.* **58**:95–99.

Hong, S. L., and Deykin, D., 1981, The activation of phosphatidylinositol-hydrolyzing phospholipase A_2 during prostaglandin synthesis in transformed mouse BALB/3T3 cells, *J. Biol. Chem.* **256**:5215–5219.

Hooper, S. B., Watkins, W. B., and Thorburn, G. D., 1986, Oxytocin, oxytocin-associated neurophysin, and prostaglandin $F_{2\alpha}$ concentrations in the utero–ovarian vein of pregnant and nonpregnant sheep, *Endocrinology* **119**:2590–2597.

Horton, E. W., and Poyser, N. L., 1976, Uterine luteolytic hormone: A physiological role for prostaglandin $F_{2\alpha}$, *Physiol. Rev.* **56**:595–651.

Hsu, C. J., McCormack, S., and Sanborn, B. M., 1985, The effect of relaxin on cyclic adenosine 3′,5′-monophosphate concentrations in rat myometrial cells in culture, *Endocrinology* **116**:2029–2035.

Husslein, P., Fuchs, A.-R., and Fuchs, F., 1981, Oxytocin and the initiation of human parturition. I. Prostaglandin release during induction of labor by oxytocin, *Am. J. Obstet. Gynecol.* **141**:688–693.

Husslein, P., Kofler, E., Rasmussen, A. B., Sumulong, L., Fuchs. A.-R., and Fuchs, F., 1983, Oxytocin and the initiation of human parturition. IV. Plasma concentrations of oxytocin and 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ during induction of labor by artificial rupture of the membranes, *Am. J. Obstet. Gynecol.* **147**:503–507.

Huszar, G., 1986, Cellular regulation of myometrial contractility and essentials of tocolytic therapy, in: *The Physiology and Biochemistry of the Uterus in Pregnancy and Labor* (G. Huszar, ed.), CRC Press, Boca Raton, FL, pp. 107–126.

Huszar, G., Cabrol, D., and Naftolin, F., 1986, The relationship between myometrial contractility and cervical maturation in pregnancy and labor, in: *The Physiology and Biochemistry of the Uterus in Pregnancy and Labor* (G. Huszar, ed.), CRC Press, Boca Raton, FL, pp. 297–306.

Ichida, S., Tokunaga, H., Oda, Y., Fujita, N., Hirata, A., and Hata, T., 1983, Increase of serotonin receptors in rat uterus induced by estradiol, *J. Biol. Chem.* **258**:13438–13443.

Irvine, R. F., 1982, How is the level of free arachidonic acid controlled in mammalian cells? *Biochem. J.* **204**:3–16.

Janis, R. A., and Triggle, D. J., 1986, Effects of calcium channel antagonists on the myometrium, in: *The Physiology and Biochemistry of the Uterus in Pregnancy and Labor* (G. Huszar ed.), CRC Press, Boca Raton, FL, pp. 201–223.

Järvinen, P. A., Luukkainen, T., and Väistö, L., 1965, The effect of oestrogen treatment on myometrial activity in late pregnancy, *Acta Obstet. Gynecol. Scand.* **44**:258–264.

Johnson, M., Jessup, R., Jessup, S., and Ramwell, P. W., 1974, Correlation of prostaglandin E_1 receptor binding with evoked uterine contraction: Modification by disulfide reduction, *Prostaglandins* **6**:433–449.

Jones, S. A., and Summerlee, A. J. S., 1987, Effects of chronic infusion of porcine relaxin on oxytocin release in lactating rats, *J. Endocrinol.* **114**:241–246.

Joseph, M. K., Fernstrom, M. A., and Soloff, M. S., 1982, Switching of β- to α-tubulin phosphorylation in uterine smooth muscle of parturient rats, *J. Biol. Chem.* **257**:11728–11733.

Judson, D. G., Pay, S., and Bhoola, K. D., 1980, Modulation of cyclic AMP in isolated rat uterine tissue slices by porcine relaxin, *J. Endocrinol.* **87**:153–159.

Kamm, K. E., and Stull, J. T., 1985, The function of myosin and myosin light chain kinase phosphorylation in smooth muscle, *Annu. Rev. Pharmacol. Toxicol.* **25**:593–620.

Kao, C. Y., 1976, Electrophysiological properties of uterine smooth muscle, in: *Biology of the Uterus* (R. M. Wynn, ed.), Plenum Press, New York, pp. 423–496.

Karim, S., 1971, Action of prostaglandin in the pregnant woman, *Ann. N.Y. Acad. Sci.* **180**:483–498.

Karim, S. M. M., and Devlin, J., 1967, Prostaglandin content of amniotic fluid during pregnancy and labour, *J. Obstet. Gynaecol. Br. Commonw.* **74**:230–234.

Keirse, M. J. N. C., 1983, Prostaglandins during human parturition, in: *Initiation of Parturition: Prevention of Prematurity* (P. C. MacDonald and J. Porter, eds.), Ross Laboratories, Columbus, OH, pp. 137–144.

Keirse, M. J. N. C., and Turnbull, A. C., 1973, E Prostaglandins in amniotic fluid during late pregnancy and labour, *J. Obstet. Gynaecol. Br. Commonw.* **80**:970–973.

Keirse, M. J. N. C., and Turnbull, A. C., 1975, Metabolism of prostaglandins within the pregnant uterus, *Br. J. Obstet. Gynaecol.* **82**:887–893.

Keirse, M. J. N. C., Mitchell, M. D., and Turnbull, A. C., 1977, Changes in prostaglandin F and 13,14-dihydro-15-keto-prostaglandin F concentrations in amniotic fluid at the onset of and during labour, *Br. J. Obstet. Gynaecol.* **84**:743–746.

Kertiles, L. P., and Anderson, L. L., 1979, Effect of relaxin on cervical dilatation, parturition and lactation in the pig, *Biol. Reprod.* **21**:57–68.

Khan-Dawood, F. S., and Dawood, M., 1984, Estrogen and progesterone receptor and hormone levels in human myometrium and placenta in term pregnancy, *Am. J. Obstet. Gynecol.* **150**:501–505.

Kinoshita, K., Satoh, K., and Sakamoto, S., 1977, Biosynthesis of prostaglandin in human decidua, amnion, chorion, and villi, *Endocrinol. Jpn.* **24**:343–350.

Klopper, A. I., and Dennis, L. K., 1962, Effect of oestrogens on myometrial contractions, *Br. Med. J.* **2**:1157–1159.

Kolesnick, R. N., Musacchio, I., Thaw, C., and Gershengorn, M. C., 1984, Arachidonic acid mobilizes calcium and stimulates prolactin secretion from GH_3 cells, *Am. J. Physiol.* **246**:E458–E462.

Krall, J. F., Mori, H., Tuck, M. L., LeShon, S. L., and Korenman, S. G., 1978, Demonstration of adrenergic catecholamine receptors in rat myometrium and their regulation by sex steroids, *Life Sci.* **23**:1073–1081.

Kroc, R. L., Steinetz, B. G., and Beach, V. L., 1959, The effects of estrogens, progestagens, and relaxin in pregnant and nonpregnant laboratory rodents, *Ann. N.Y. Acad. Sci.* **75**:942–980.

Kuriyama, H., and Suzuki, H., 1976, Effects of prostaglandin E_2 and oxytocin on the electrical activity of hormone-treated and pregnant rat myometria, *J. Physiol. (Lond.)* **260**:335–349.

Lackritz, R., Tulchinsky, D., Ryan, K. J., and Levine, L., 1978, Plasma prostaglandin metabolites in human labor, *Am. J. Obstet. Gynecol.* **131**:484–489.

Lange, A. P., Secher, N. J., Westergaard, J. G., and Skovgård, I. B., 1982, Prelabor evaluation of inducibility, *Obstet, Gynecol,* **60**:137–147.

Leake, R. D., Weitzman, R. E., Glatz, T. H., and Fisher, D. A., 1981, Plasma oxytocin concentrations in men, nonpregnant women, and pregnant women before and during spontaneous labor, *J. Clin. Endocrinol. Metab.* **53**:730–733.

Leaver, H. A., and Richmond, D. H., 1984, The effects of oxytocin, estrogen, calcium ionophore A23187 and hydrocortisone on prostaglandin $F_{2\alpha}$ and 6-oxo-prostaglandin $F_{1\alpha}$ production by cultured human endometrial and myometrial explants, *Prostaglandins Leukotrienes Med.* **13**:179–196.

Leaver, H. A., and Seawright, A., 1982, Controls of endometrial prostaglandin output *in vitro* during the estrous cycle of the guinea pig: influence of estradiol 17β, progesterone, oxytocin and calcium ionophore A23187, *Prostaglandins Leukotrienes Med.* **9**:657–668.

Levine, L., and Gutierrez-Cernosek, R. M. C., 1973, Levels of 13,14-dihydro-15-keto-$PGF_{2\alpha}$ in biological fluids as measured by radioimmunoassay, *Prostaglandins* **3**:785–804.

Lewis, R. B., and Schulman, J. D., 1973, Influence of acetylsalicylic acid, an inhibitor of prostaglandin synthesis, on the duration of human gestation and labour, *Lancet* **2**:1159–1161.

Liggins, G. C., Fairclough, R. J., Grieves, S. A., Kendall, J. Z., and Knox, B. S., 1973, The mechanism of initiation of parturition in the ewe, *Recent Prog. Horm. Res.* **29**:111–159.

Lopez Bernal, A., Hansell, D. J., Alexander, S., and Turnbull, A. C., 1987, Prostaglandin E production by amniotic cells in relation to term and preterm labour, *Br. J. Obstet. Gynaecol.* **94**:864–869.

Lumsden, M. A., and Baird, D. T., 1986, The effect of intrauterine administration of prostacyclin on the contractility of the non-pregnant uterus *in vivo, Prostaglandins* **31**:1011–1022.

Lye, S. J., and Challis, J. R. G., 1982, Inhibition by PGI_2 of myometrial activity *in vivo* in non-pregnant ovariectomized sheep, *J. Reprod. Fertil.* **66**:311–315.

Maathuis, J. B., and Kelly, R. W., 1978, Concentrations of prostaglandins $F_{2\alpha}$ and E_2 in the endometrium throughout the human menstrual cycle, after the administration of clomiphene or an oestrogen–progestogen pill and in early pregnancy, *J. Endocrinol.* **77**:361–371.

MacDonald, P. C., Schultz, F. M., Duenhoelter, J. H., Gant, N. F., Jimenez, J. M., Pritchard, J. A., Porter, J. C., and Johnston, J. M., 1974, Initiation of human parturition. I. Mechanism of action of arachidonic acid, *Obstet. Gynecol.* **44**:629–636.

MacKenzie, I. Z., and Embrey, M. P., 1977, Cervical ripening with intravaginal prostaglandin E_2 gel, *Br. Med. J.* **2**:1381–1384.

MacKenzie, I. Z., and Mitchell, M. D., 1981, Serial determinations of prostaglandin E in amniotic fluid following the vaginal administration of a prostaglandin E_2 gel, *Prostaglandins Med.* **7**:43–47.

MacLennan, A. H., Green, R. C., Bryant-Greenwood, G. D., Greenwood, F. C., and Seamark, R. F., 1980, Ripening of the human cervix and induction of labour with purified porcine relaxin, *Lancet* **1**:220–223.

Majerus, P. W., 1983, Arachidonate metabolism in vascular disorders, *J. Clin. Invest.* **72**:1521–1525.

Manzai, M., and Liggins, G. C., 1984, Inhibitory effects of dispersed human amnion cells on production of prostaglandin E and F by endometrial cells, *Prostaglandins* **28**:297–307.

Marc, S., Leiber, D., and Harbon, S., 1986, Carbachol and oxytocin stimulate the generation of inositol phosphates in the guinea pig myometrium, *FEBS Lett.* **201**:9–14.

Marshall, J. M., 1959, Effects of estrogen and progesterone on single uterine muscle fiber in the rat, *Am. J. Physiol.* **197**:935–942.

Marshall, J. M., 1974, Effects of neurohypophysial hormones on the myometrium, in: *The Pituitary Gland and its Neuroendocrine Control,* Part 1, *Handbook of Physiology,* Section 7: *Endocrinology,* Vol. IV (R. O.

Gr... (E. B. Astwood, E. Knobil, W. H. Sawyer, and ... R. Geiger, eds.), American Physiological Society, Washington, pp. 469–492.

McCoshen, J. A., Johnson, K. A., Dubin, N. H., and Ghodgaonkar, R. B., 1987, Prostaglandin E_2 release on the fetal and maternal sides of the amnion and chorion–decidua before and after term labor, *Am. J. Obstet. Gynecol.* **156**:173–178.

McCracken, J. A., Carlson, J. C., Glew, M. E., Goding, J. R., Baird, D. T., Green, K., and Samuelsson, B., 1972, Prostaglandin $F_{2\alpha}$ identified as luteolytic hormone in sheep, *Nature (New Biol)* **238**:129–134.

McPhail, L. C., Clayton, C. C., and Snyderman, R., 1984, A potential second messenger role for unsaturated fatty acids: Activation of Ca^{2+}-dependent protein kinase, *Science* **224**:622–625.

Mercado-Simmen, R. C., Bryant-Greenwood, G. D., and Greenwood, F. C., 1980, Relaxin receptor in the rat myometrium: Regulation by estrogen and relaxin, *Endocrinology* **110**:220–226.

Mercado-Simmen, R. C., Goodwin, B., Ueno, M. S., Yamamoto, S. Y., and Bryant-Greenwood, G. D., 1982, Relaxin receptors in the myometrium and cervix of the pig, *Biol. Reprod.* **26**:120–128.

Metz, S. A., Draznin, B., Sussman, K. E., and Leitner, J. W., 1987, Unmasking of arachidonate-induced insulin release by removal of extracellular calcium, *Biochem. Biophys. Res. Commun.* **142**:251–258.

Milewich, L., Gant, N. F., Schwarz, B., Chen, G. T., and MacDonald, P. C., 1977, Initiation of human parturition. VIII. Metabolism of progesterone by fetal membranes of early and late human gestation, *Obstet. Gynecol.* **50**:45–48.

Miller, M. D., and Marshall, J. M., 1965, Uterine response to nerve stimulation; Relation to hormonal status and catecholamines, *Am. J. Physiol.* **209**:859–865.

Miller, W. C., and Moore, J. B., Jr., 1984, High affinity binding sites for [^{3}H]-nitrendipine in rabbit uterine smooth muscle, *Life Sci.* **34**:1717–1724.

Mitchell, B., Cruickshank, B., McLean, D., and Challis, J., 1982, Local modulation of progesterone production in human fetal membranes, *J. Clin. Endocrinol. Metab.* **55**:1237–1239.

Mitchell, B. F., Challis, J. R. G., and Lukash, L., 1987, Progesterone synthesis by human amnion, chorion, and decidua at term, *Am. J. Obstet. Gynecol.* **157**:349–353.

Mitchell, M. D., Flint, A. P. F., and Turnbull, A. C., 1975a, Stimulation by oxytocin of prostaglandin F levels in uterine venous effluent in pregnant and puerperal sheep, *Prostaglandins* **9**:47–56.

Mitchell, M. D., Keirse, M. J. N. C., Anderson, A. B. M., and Turnbull, A. C., 1975b, Evidence for a local control of prostaglandins within the pregnant human uterus, *Br. J. Obstet. Gynaecol.* **84**:35–38.

Mitchell, M. D., Flint, A. P. F., and Turnbull, A. C., 1976a, Plasma concentrations of 13,14-dihydro-15-keto-prostaglandin F during pregnancy in sheep, *Prostaglandins* **11**:319–329.

Mitchell, M. D., Patrick, J. E., Robinson, J. S., Thorburn, G. D., and Challis, J. R. G., 1976b, Prostaglandins in the plasma and amniotic fluid of rhesus monkeys during pregnancy and after intra-uterine foetal death, *J. Endocrinol.* **71**:67–76.

Mitchell, M. D., Flint, A. P. F., Bibby, J., Brunt, J., Arnold, J. M., Anderson, A. B. M., and Turnbull, A. C., 1977, Rapid increases in plasma prostaglandin concentrations after vaginal examination and amniotomy, *Br. Med. J.* **2**:1183–1185.

Mitchell, M. D., Bibby, J., Hicks, B. R., and Turnbull, A. C., 1978, Specific production of prostaglandin E by human amnion *in vitro*, *Prostaglandins* **15**:377–382.

Mitchell, M. D., Strickland, D. M., Brennecke, S. P., and Saeed, S. A., 1983, New aspects of arachidonic acid metabolism in human parturition, in: *Initiation of Parturition: Prevention of Prematurity* (P. C. MacDonald and J. Porter, eds.), Ross Laboratories, Columbus, OH, pp. 145–150.

Mitchell, M. D., MacDonald, P. C., and Casey, M. L., 1984, Stimulation of prostaglandin E_2 synthesis in human amnion cells maintained in monolayer culture by a substance(s), in amniotic fluid, *Prostaglandins Leukotrienes Med.* **15**:399–407.

Montrucchio, G., Alloatti, G., Tetta, C., Roffinello, C., Emanuelli, G., and Camusi, G., 1986, *In vitro* contractile effect of platelet-activating factor on guinea pig myometrium, *Prostaglandins* **32**:539–554.

Mukku, V. R., and Stancel, G. M., 1985, Regulation of epidermal growth factor receptor by estrogen, *J. Biol. Chem.* **260**:9820–9824.

Murphy, B. E. P., 1982, Human fetal serum cortisol levels related to gestational age: evidence of midgestational fall and a steep late rise, independent of sex or mode of delivery, *Am. J. Obstet; Gynecol.* **144**:276–282.

Musah, A. I., Schwabe, C., Willham, R. L., and Anderson, L. L., 1986, Relaxin on induction of parturition in beef heifers, *Endocrinology* **118**:1476–1482.

Nagata, I., Sunaga, H., Furuya, K., Makimura, N., and Kato, K., 1987, Changes in the plasma prostaglandin $F_{2\alpha}$ metabolite before and during spontaneous labor and labor induced by amniotomy, oxytocin, and PGE_2, *Endocrinol. Jpn.* **34**:153–159.

Nakla, S., Skinner, K., Mitchell, B. F., and Challis, J. R. G., 1986, Changes in prostaglandin transfer across human fetal membranes obtained after spontaneous labor. *Am. J. Obstet. Gynecol.* **155**:1337–1341.

Nieder, J., and Augustin, W., 1983, Increase of prostaglandin E and F equivalents in amniotic fluid during late pregnancy and rapid PGF elevation after cervical dilatation, *Prostaglandins Leukotrienes Med.* **12**:289–297.

Niesert, S., Christopherson, W., Korte, K., Mitchell, M. D., MacDonald, P. C., and Casey, M. L., 1986, Prostaglandin E_2 9-ketoreductase activity in human decidua vera tissue, *Am. J. Obstet. Gynecol.* **155**:1348–1352.

Nishihara, J., Ishibashi, T., Imai, Y., and Muramatsu, T., 1984, Mass spectrometric evidence for the presence of platelet-activating factor (1-O-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) in human amniotic fluid during labor, *Lipids* **19**:907–910.

Nishikori, K., Weisbrodt, N. W., Sherwood, O. D., and Sanborn, B. M., 1983, Effects of relaxin on rat uterine myosin light chain kinase activity and myosin light chain phosphorylation, *J. Biol. Chem.* **258**:2468–2474.

Northover, B. J., 1972, The effects of indomethacin on calcium, sodium, potassium and magnesium fluxes in various tissues of the guinea-pig, *Br. J. Pharmacol.* **45**:651–659.

Novy, M. J., 1983, Endocrine control of parturition in rhesus monkeys, in: *Initiation of Parturition: Prevention of Prematurity* (P. C. MacDonald and J. Porter, eds.), Ross Laboratories, Columbus, OH, pp. 62–71.

Novy, M. J., Cook, M. J., and Manaugh, L., 1974, Indomethacin block of normal onset of parturition in primates, *Am. J. Obstet. Gynecol.* **118**:412–416.

O'Grady, J. P., Caldwell, B. V., Auletta, F. J., and Speroff, L., 1972, The effects of an inhibitor of prostaglandin synthesis (indomethacin) on ovulation, pregnancy, and pseudopregnancy in the rabbit, *Prostaglandins* **1**:97–106.

Okazaki, T., Casey, M. L., Okita, J. R., MacDonald, P. C., and Johnston, J. M., 1981, Initiation of human parturition. XII. Biosynthesis and metabolism of prostaglandins in human fetal membranes and uterine decidua, *Am. J. Obstet. Gynecol.* **139**:373–381.

Olson, D. M., Skinner, K., and Challis, J. R. G., 1983a, Prostaglandin output in relation to parturition by cells dispersed from human intrauterine tissues, *J. Clin. Endocrinol. Metab.* **57**:694–699.

Olson, D. M., Opavsky, M. A., and Challis, J. R. G., 1983b, Prostaglandin synthesis by human amnion is dependent upon extracellular calcium, *Can. J. Physiol. Pharmacol.* **61**:1089–1092.

Omini, C., Folco, G. C., Pasargiklian, R., Fano, M., and Berti, F., 1979, Prostacyclin (PGI_2) in pregnant human uterus, *Prostaglandins* **17**:113–120.

Padayachi, T., Norman, R. J., Reddi, K., Schweni, M., Philpott, H., and Joubert, S. M., 1986, Changes in amniotic fluid prostaglandins with oxytocin-induced labor, *Obstet. Gynecol.* **68**:610–613.

Pavlik, E. J., and Coulson, P. B., 1976, Modulation of estrogen receptors in four different target tissues: Differential effects of estrogen vs. progesterone, *J. Steroid Biochem.* **7**:369–376.

Pearce, D. J., 1977, Pre-induction priming of the uterine cervix with oral prostaglandin E_2 and a placebo, *Prostaglandins* **14**:571–576.

Pearlmutter, A. F., and Soloff, M. S., 1979, Characterization of the metal ion requirement for oxytocin–receptor interaction in rat mammary gland membranes, *J. Biol. Chem.* **254**:3899–3906.

Perry, G., Siegal, B., and Held, B., 1977, Second trimester abortion: Single dose intra-amniotic injection of prostaglandin $F_{2\alpha}$ with intraveous oxytocin augmentation, *Prostaglandins* **13**:987–994.

Pharriss, B. B., and Wyngarden, L. J., 1969, The effect of prostaglandin $F_{2\alpha}$ on the progestogen content of ovaries from pseudopregnant rats, *Proc. Soc. Exp. Biol. Med.* **130**:92–94.

Pinto, R. M., Fisch, L., Schwarcz, R. L., and Montuori, E., 1964, Action of estradiol upon uterine contractility and the milk ejecting effect in pregnant women, *Am. J. Obstet. Gynecol.* **90**:99–107.

Pinto, R. M., Leon, C., Mazzocco, N., and Scasserra, V., 1967, Action of estradiol-17β at term and at onset of labor, *Am. J. Obstet. Gynecol.* **98**:540–546.

Piper, P., and Vane, J., 1971, The release of prostaglandins from lungs and other tissues, *Ann. N.Y. Acad. Sci.* **180**:363–385.

Porter, D. G., Downing, S. J., and Bradshaw, J. M. C., 1979, Relaxin inhibits spontaneous and prostaglandin-driven myometrial activity in anaesthetized rats, *J. Endocrinol.* **83**:183–192.

Porter, D. G., Downing, S. J., and Bradshaw, J. M. C., 1981, Inhibition of oxytocin- or prostaglandin $F_{2\alpha}$-driven myometrial activity by relaxin in the rat is oestrogen dependent, *J. Endocrinol.* **89**:399–404.

Poyser, N. L., Horton, E. W., Thompson, C. J., and Los, M., 1971, Identification of prostaglandin $F_{2\alpha}$ released by distention of guinea-pig uterus *in vitro*, *Nature* **230**:526–528.

Rath, W., Adelmann-Grill, B. C., Pieper, U., and Kuhn, W., 1987, The role of collagenases and proteases in prostaglandin-induced cervical ripening, *Prostaglandins* **34**:119–127.

Raziano, J., Ferin, M., and Vande Wiele, R. L., 1972, Effects of antibodies to estradiol-17β and to progesterone on nidation and pregnancy in rats, *Endocrinology* **90**:1133–1138.

Reddi, K., Kambaran, S. R., Norman, R. J., Joubert, S. M., and Philpott, H., 1984, Abnormal concentrations of prostaglandins in amniotic fluid during delayed labour in multigravid patients, *Br. J. Obstet. Gynaecol.* **91**:781–787.

Rehnströ[illegible], Ishikawa, M., Fuchs, F., and Fuchs, A.-R., [illegible], Stimulation of myometrial and decidual prostaglandin production by amniotic fluid from term, but not midterm pregnancies, *Prostaglandins* **26**:973–981.

Reynolds, S. R. M., Hellman, L. M., and Bruns, P., 1948, Patterns of uterine contractility in women during pregnancy, *Obstet. Gynecol. Surv.* **3**:629–646.

Riemer, R. K., Goldfien, A. C., Goldfien, A., and Roberts, J. M., 1986, Rabbit uterine oxytocin receptors and *in vitro* contractile response: Abrupt changes at term and the role of eicosanoids, *Endocrinology* **119**:699–709.

Riley, S. C., and Poyser, N. L., 1987, Prostaglandin production by the guinea-pig endometrium: Is calcium necessary? *J. Endocrinol.* **113**:463–471.

Roberts, J. M., Insel, P. A., and Goldfien, A., 1981, Regulation of myometrial adrenoreceptors and adrenergic response by sex steroids, *Mol. Pharmacol.* **20**:52–58.

Roberts, J. S., and McCracken, J. A., 1976, Does prostaglandin $F_{2\alpha}$ released from the uterus by oxytocin mediate the oxytocic action of oxytocin? *Biol. Reprod.* **15**:457–463.

Roberts, J. S., Barcikowski, B., Wilson, L., Skarnes, R. C., and McCracken, J. A., 1975, Hormonal and related factors affecting the release of prostaglandin $F_{2\alpha}$ from the uterus, *J. Steroid Biochem.* **6**:1091–1097.

Roberts, J. S., McCracken, J. A., Gavagan, J. E., and Soloff, M. S., 1976, Oxytocin-stimulated release of prostaglandin $F_{2\alpha}$ from ovine endometrium *in vitro:* Correlation with estrous cycle and oxytocin–receptor binding, *Endocrinology* **99**:1107–1114.

Robinson, J. S., Chapman, R. L. K., Challis, J. R. G., Mitchell, M. D., and Thorburn, G. D., 1978, Administration of extra-amniotic arachidonic acid and the suppression of uterine prostaglandin synthesis during pregnancy in the rhesus monkey, *J. Reprod. Fertil.* **54**:369–373.

Romero, R., Lafreniere, D., Hobbins, J. C., and Mitchell, M. D., 1987, A soluble product from human decidua inhibits prostaglandin production by human amnion, *Prostaglandins Leukotrienes Med.* **30**:29–35.

Saeed, S. A., and Mitchell, M. D., 1982, Stimulants of prostaglandin biosynthesis in human fetal membranes, uterine decidua vera and placenta, *Prostaglandins* **24**:475–484.

Saeed, S. A., McDonald-Gibson, W. J., Cuthbert, J., Copas, J. L., Schneider, C., Gardiner, P. J., Butt, N. M., and Collier, H. O. J., 1977, Endogenous inhibitor of prostaglandin synthetase, *Nature* **270**:32–36.

Saeed, S. A., Strickland, D. M., Young, D. C., Dang, A., and Mitchell, M. D., 1982, Inhibition of prostaglandin synthesis by human amniotic fluid: Acute reduction in inhibitory activity of amniotic fluid obtained during labor, *J. Clin. Endocrinol. Metab.* **55**:801–803.

Sakai, K., Yamaguchi, T., Morita, S., and Uchida, M., 1983, Agonist-induced contraction of rat myometrium in Ca-free solution containing Mn, *Gen. Pharmacol.* **14**:391–400.

Saldana, L. R., Schulman, H., Yang, W. H., Cunningham, M. A., and Randolf, G., 1974, Midtrimester abortion by prostaglandin impact, *Obstet. Gynecol.* **44**:579–585.

Salomy, M., Halbrecht, I., and London, R., 1975, Mid-trimester abortion with intra-amniotic prostaglandin $F_{2\alpha}$ and intravenous oxytocin infusion, *Prostaglandins* **9**:271–279.

Samuelsson, B., Granström, E., Gréen, K., Hamberg, M., and Hammarström, S., 1975, Prostaglandins, *Annu. Rev. Biochem.* **44**:669–695.

Samuelsson, B., Goldyne, M., Granström, E., Hamberg, M., Hammarström, S., and Malmsten, C., 1978, Prostaglandins and thromboxanes, *Annu. Rev. Biochem.* **47**:997–1029.

Sanborn, B. M., 1986, The role of relaxin in uterine function, in: *The Physiology and Biochemistry of the Uterus in Pregnancy and Labor* (G. Huszar, ed.), CRC Press, Boca Raton, FL, pp. 225–238.

Sanborn, B. M., Kuo, H. S., Weisbrodt, N. W., and Sherwood, O. D., 1980, The interaction of relaxin with the rat uterus. I. Effect on cyclic nucleotide levels and spontaneous contractile activity, *Endocrinology* **106**:1210–1215.

Sawyer, W. H., Frieden, E. H., and Martin, A. S., 1953, *In vitro* inhibition of spontaneous contractions of the rat uterus by relaxin-containing extracts of sow ovaries, *Am. J. Physiol.* **178**:547–552.

Schatz, F., Markiewicz, L., and Gurpide, E., 1984, Effects of estriol on $PGF_{2\alpha}$ output by cultures of human endometrium and endometrial cells, *J. Steroid Biochem.* **20**:999–1003.

Schatz, F., Markiewicz, L., and Gurpide, E., 1987, Differential effects of estradiol, arachidonic acid, and A23187 on prostaglandin $F_{2\alpha}$ output by epithelial and stromal cells of human endometrium, *Endocrinology* **120**:1465–1471.

Schillinger, E., and Prior, G., 1976, Characteristics of prostaglandin receptor sites in human uterine tissue, *Adv. Prostaglandin Thromboxane Res.* **1**:259–263.

Schirar, A., Capponi, A., and Catt, K. J., 1980, Regulation of uterine angiotensin II receptors by estrogen and progesterone, *Endocrinology* **106**:5–12.

Schofield, B. M., 1957, The hormonal control of myometrial function during pregnancy, *J. Physiol. (Lond.)* **138**:1–10.

Schofield, B. M., 1964, Myometrial activity in the pregnant guinea-pig, *J. Endocrinol.* **30**:347–354.

Schofield, B. M., 1968, Parturition, in: *Advances in Reproductive Physiology*, Vol. 3 (A. McLaren, ed.), Academic Press, New York, pp. 9–32.

Schrey, M. P., Read, A. M., and Steer, P. J., 1986, Oxytocin and vasopressin stimulate inositol phosphate production in human gestational myometrium and decidual cells, *Biosci. Rep.* **6**:613–619.

Schwabe, C., Steinetz, B., Weiss, G., Segaloff, A., McDonald, J. K., O'Byrne, E., Hochman, J., Carriere, B., and Goldsmith, L., 1978, Relaxin, *Recent Prog. Horm. Res.* **34**:123–211.

Schwalm, H., and Dubrauszky, V., 1966, The structure of the musculature of the human uterus–muscles and connective tissue, *Am. J. Obstet. Gynecol.* **94**:391–404.

Scott, R. S., and Rennie, P. I. C., 1970, Factors controlling the lifespan of the corpora lutea in the pseudopregnant rabbit, *J. Reprod. Fertil.* **23**:415–422.

Sellers, S. M., Hodgson, H. T., Mitchell, M. D., Anderson, A. B. M., and Turnbull, A. C., 1982, Raised prostaglandin levels in the third stage of labor, *Am. J. Obstet. Gynecol.* **144**:209–212.

Seppala, M., Kajanona, P., Widholm, O., and Vara, P., 1972, Prostaglandin–oxytocin abortion: A clinical trial on intra-amniotic prostaglandin $F_{2\alpha}$ in combination with intravenous oxytocin, *Prostaglandins* **2**:311–319.

Sharma, S. C., and Fitzpatrick, R. J., 1974, Effect of oestradiol-17β and oxytocin on prostaglandin F alpha release in the anoestrus ewe, *Prostaglandins* **6**:97–105.

Sheldrick, E. L., Mitchell, M. D., and Flint, A. P. F., 1980, Delayed luteal regression in ewes immunized against oxytocin, *J. Reprod. Fertil.* **59**:37–42.

Sherwood, O. D., Nara, B. S., Crnekovic, V. E., and First, N. L., 1969, Relaxin concentrations in pig plasma after the administration of indomethacin and prostaglandin $F_{2\alpha}$ during late pregnancy, *Endocrinology* **104**:1716–1721.

Skinner, K. A., and Challis, J. R. G., 1985, Changes in the synthesis and metabolism of prostaglandins by human fetal membranes and decidua at labor, *Am. J. Obstet. Gynecol.* **151**:519–523.

Skinner, S J. M., Liggins, G. C., Wilson, T., and Neale, G., 1984, Synthesis of prostaglandin F by cultured human endometrial cells, *Prostaglandins* **27**:821–838.

Soloff, M. S., and Alexandrova, M., 1981, Parturition, lactation and the regulation of oxytocin receptors, in: *Reproductive Processes and Contraception* (K. W. McKerns, ed.), Plenum Press, New York, pp. 281–303.

Soloff, M. S., and Grzonka, Z., 1986, Effects of manganese on relative affinities of receptors for oxytocin analogues. Binding studies with rat myometrial and mammary gland membranes, *Endocrinology* **119**:1564–1569.

Soloff, M. S., and Swartz, T. L., 1974, Characterization of a proposed oxytocin receptor in the uterus of the rat and sow, *J. Biol. Chem.* **249**:1376–1381.

Soloff, M. S., and Sweet, P., 1982, Oxytocin inhibition of $(Ca^{2+} + Mg^{2+})$ATPase activity in rat myometrial plasma membranes, *J. Biol. Chem.* **257**:10687–10693.

Soloff, M. S., Alexandrova, M., and Fernstrom, M. J., 1979, Oxytocin receptors: Triggers for parturition and lactation? *Science* **204**:1313–1315.

Soloff, M. S., Fernström, M. A., Periyasamy, S., Soloff, S., Baldwin, S., and Wieder, M., 1983, Regulation of oxytocin receptor concentration in rat uterine explants by estrogen and progesterone, *Can. J. Biochem. Cell Biol.* **61**:625–630.

Spellacy, W. N., Cruz, A. C., Birk, S. A., and Buhi, W. C., 1979, Treatment of premature labor with ritodrine: A randomized controlled study, *Obstet. Gynecol.* **54**:220–223.

Speroff, L., Caldwell, B. V., Brock, W. A., Anderson, G. G., and Hobbins, J. C., 1972, Hormone levels during prostaglandin $F_{2\alpha}$ infusions for therapeutic abortion, *J. Clin. Endocrinol. Metab.* **34**:531–536.

Strauss, J. F. III, and Stambaugh, R. L., 1974, Induction of 20α-hydroxysteroid dehydrogenase in rat corpora lutea of pregnancy by prostaglandin $F_{2\alpha}$ *Prostaglandins* **5**:73–85.

Strauss, J. F. III, Sokoloski, J., Caploe, P., Duffy, P., Mintz, G., and Stambaugh, R. L., 1975, On the role of prostaglandins in parturition in the rat, *Endocrinology* **96**:1040–1043.

Strickland, D. M., Saeed, S. A., Casey, M. L., and Mitchell, M. D., 1983, Stimulation of prostaglandin biosynthesis by urine of the human fetus may serve as a trigger for parturition, *Science* **220**:521–522.

Stys, S. J., 1986, Endocrine regulation of cervical functions during pregnancy, in: *The Physiology and Biochemistry of the Uterus in Pregnancy and Labor* (G. Huszar, ed.), CRC Press, Boca Raton, FL, pp. 281–295.

Summerlee, A. J. S., O'Byrne, K. T., Paisley, A. C., Breeze, M. F., and Porter, D. G., 1984, Relaxin affects the central control of oxytocin release, *Nature* **309**:372–374.

Szlachter, N., O'Byrne, E., Goldsmith, L., Steinetz, B. G., and Weiss, G., 1980, Myometrial inhibiting

ac . y of relaxin-containing extracts of human corpora lutea of pregnancy, *Am. J. Obstet. Gynecol.* **136**:584–586.

Takahashi, K., Diamond, F., Bieniarz, J., Yen, H., and Burd, L., 1980, Uterine contractility and oxytocin sensitivity in preterm, term, and postterm pregnancy, *Am. J. Obstet. Gynecol.* **136**:774–779.

Tansey, T. R., and Padykula, H. A., 1978, Cellular responses to experimental inhibition of collagen degradation in the postpartum rat uterus, *Anat. Rec.* **191**:287–309.

Taylor, M. J., Webb, R., Mitchell, M. D., and Robinson, J. S., 1982, Effect of progesterone withdrawal in sheep during late pregnancy, *J. Endocrinol.* **92**:85–93.

Tetta, C., Montrucchio, G., Alloatti, G., Roffinello, C., Emanuelli, G., Benedetto, C., Camussi, G., and Massobrio, M., 1986, Platelet-activating factor contracts human myometrium *in vitro, Proc. Soc. Exp. Biol. Med.* **183**:376–381.

Theobald, G. W., 1959, The choice between death from postmaturity or prolapsed cord and life from induction of labour, *Lancet* **1**:59–65.

Theobald, P. W., Rath, W., Kühnle, H.,and Kuhn, W., 1982, Histologic and electron-microscopic examinations of collagenous connective tissue of the non-pregnant cervix, the pregnant cervix, and the pregnant prostaglandin-treated cervix, *Arch. Gynecol.* **231**:241–245.

Thorbert, G., 1978, Regional changes in structure and function of adrenergic nerves in guinea-pig uterus during pregnancy, *Acta Obstet. Gynecol. Scand. [Suppl.]* **79**:1–32.

Thorbert, G., Alm, P., Björklund, A. B., Owman, C., and Sjöberg, N.-O., 1979, Adrenergic innervation of the human uterus: Disappearance of the transmitter and transmitter-forming enzymes during pregnancy, *Am. J. Obstet. Gynecol.* **135**:223–226.

Thorburn, G. D., and Challis, J. R. G., 1979, Endocrine control of parturition, *Physiol. Rev.* **59**:863–918.

Thorburn, G. D., Challis, J. R. G., and Robinson, J. S., 1977, Endocrine control of parturition, in: *Biology of the Uterus* (R. M. Wynn, ed.), Plenum Press, New York, pp. 653–732.

Touqui, L., Rothhut, B., Shaw, A. M., Fradin, A., Vargaftig, B. B., and Russo-Marie, F., 1986, Platelet activation—a role for a 40K anti-phospholipase A_2 protein indistinguishable from lipocortin, *Nature* **321**:177–180.

Ulmsten, U., Andersson, K.-E., and Wingerup, L., 1980, Treatment of premature labor with the calcium antagonist nifedipine, *Arch. Gynecol.* **229**:1–5.

Valentine, B. H., 1977, Intravenous oxytocin and oral prostaglandin E_2 for ripening of the unfavourable cervix, *Br. J. Obstet. Gynecol.* **84**:846–854.

Vane, J. R., and Williams, K. I., 1972, Prostaglandin production contributes to the contractions of the rat isolated uterus, *Br. J. Pharmacol.* **45**:146P.

Vesin, M.-F., Khac, L. D., and Harbon, S., 1978, Modulation of intracellular adenosine cyclic 3′,5′-monophosphate and contractility of rat uterus by prostaglandins and polyunsaturated fatty acids, *Mol. Pharmacol.* **14**:24–37.

Vu Hai, M. T., Logeat, F., Warembourg, M., and Milgrom, E., 1977, Hormonal control of progesterone receptors, *Ann. N.Y. Acad. Sci.* **286**:199–209.

Wahl, L. M., Blandau, R. J., and Page, R. C., 1977, Effect of hormones on collagen metabolism and collagenase activity in the pubic symphysis ligament of the guinea pig, *Endocrinology* **100**:571–579.

Wallner, B. P., Mattaliano, R. J., Hession, C., Cate, R. L., Tizard, R., Sinclair, L. K., Foeller, C., Chow, E. P., Browning, J. L., Ramachandran, K. L., and Pepinsky, R. B., 1986, Cloning and expression of human lipocortin, a phospholipase A_2 inhibitor with potential anti-inflammatory activity, *Nature* **320**:77–81.

Walsh, S. W., Kittinger, G. W., and Novy, M. J., 1979a, Maternal peripheral concentrations of estradiol, estrone, cortisol, and progesterone during late pregnancy in rhesus monkeys (*Macaca mulatta*) and after experimental fetal anencephaly and fetal death, *Am. J. Obstet. Gynecol.* **135**:37–42.

Walsh, S. W., Norman, R. L., and Novy, M. J., 1979b, *In utero* regulation of rhesus monkey fetal adrenals: Effects of dexamethasone, adrenocorticotropin, thyrothropin-releasing hormone, prolactin, human chorionic gonadotropin, and α-melanocyte-stimulating hormone on fetal and maternal plasma steroids, *Endocrinology* **104**:1805–1813.

Walsh, S. W., Resko, J. A., Grumbach, M. M., and Novy, M. J., 1980 *In utero* evidence for a functional fetoplacental unit in rhesus monkeys, *Biol. Reprod.* **23**:264–270.

Waltman, R., Tricomi, V., and Palav, A., 1973, Aspirin and indomethacin: Effect on instillation/abortion time on mid-trimester hypertonic saline induced abortion, *Prostaglandins* **3**:47–58.

Warnock, D. H., and Csapo, A. I., 1975, Progesterone withdrawal induced by ICI 81008 in pregnant rats, *Prostaglandins* **10**:715–724.

Warrick, C., Skinner, K., Mitchell, B. F., and Challis, J. R. G., 1985, Relation between cyclic adenosine monophosphate and prostaglandin output by dispersed cells from human amnion and decidua, *Am. J. Obstet. Gynecol.* **153**:66–71.

Wiegerinck, M. A. H. M., Poortma[illegible], Donker, T. H., and Thijssen, J. H. H., 1983, [illegible] *[illegible]ivo* uptake and subcellular distribution of tritium-labeled estrogens in human endometrium, myometrium, and vagina, *J. Clin. Endocrinol. Metab.* **56**:76–86.

Wiest, W. G., Kidwell, W. R., and Balogh, K., Jr., 1968, Progesterone catabolism in the rat ovary: A regulatory mechanism for progestational protency during pregnancy, *Endocrinology* **82**:844–859.

Wikland, M., Lindblom, B., Hammarström, S., and Wiqvist, N., 1983, The effect of prostaglandin I_2 on the contractility of the term pregnant human myometrium, *Prostaglandins* **26**:905–916.

Wilhelmsson, L., Wikland, M., and Wiqvist, N., 1981, PGH_2, TXA_2 and PGI_2 have potent and differentiated actions on human uterine contractility, *Prostaglandins* **21**:277–286.

Williams, K. I., Dembinska-Kiec, A., Zmuda, A., and Gryglewski, R. J., 1978, Prostacyclin formation by myometrial and decidual fractions of the pregnant rat uterus, *Prostaglandins* **15**:343–350.

Williams, K. I., El-Tahir, K. E. H., and Marcinkiewicz, E., 1979, Dual actions of prostacyclin (PGI_2) on the rat pregnant uterus, *Prostaglandins* **17**:667–672.

Williams, L. T., and Lefkowitz, R. J., 1977, Regulation of rabbit myometrial alpha adrenergic receptors by estrogen and progesterone, *J. Clin. Invest.* **60**:815–818.

Willman, E. A., and Collins, W. P., 1978, The metabolism of prostaglandin E_2 by tissues from the human uterus and foeto-placental unit, *Acta Endocrinol. (Kbh).* **87**:632–642.

Wiqvist, N., Lundström, V., and Gréen, K., 1985, Premature labor and indomethacin, *Prostaglandins* **10**:515–526.

Wlodawer, P., Kindahl, H., and Hamberg, M., 1976, Biosynthesis of prostaglandin $F_{2\alpha}$ from arachidonic acid and prostaglandin endoperoxides in the uterus, *Biochim. Biophys. Acta* **431**:603–614.

Wolf, B. A., Turk, J., Sherman, W. R., and McDaniel, M. L., 1986, Intracellular Ca^{2+} mobilization by arachidonic acid, *J. Biol. Chem.* **261**:3501–3511.

Zarrow, M. X., Anderson, N. C., and Callantine, M. R., 1963, Failure of progesterone to prolong pregnancy in the guinea pig, *Nature* **198**:690–692.

Zeitler, P., and Handwerger, S., 1985, Arachidonic acid stimulates phosphoinositol hydrolysis and human placental lactogen release in an enriched fraction of placental cells, *Mol. Pharmacol.* **28**:549–554.

Zuckerman, H., Reiss, U., and Rubinstein, I., 1974, Inhibition of human premature labor by indomethacin, *Obstet. Gynecol.* **44**:787–792.

pyramidal cells (*20*). It remains to be determined whether all or part of the after-hyperpolarization seen in the LC is also due to an increase in Ca^{2+}-dependent G_K. Such a finding would raise the possibility that α_2-adrenoceptors operate through a Ca^{2+}-dependent mechanism to hyperpolarize LC cells, as has been suggested for sympathetic neurons (*18*).

Intracellular recordings from LC neurons in a brain slice preparation have shown that opiates and opioid peptides produce a naloxone-reversible hyperpolarization of membrane potential associated with an increase in membrane conductance (*21*). These opiate-induced membrane effects resemble those we have observed with clonidine. Despite these similarities, clonidine and the opiates have been shown to act at different receptors in the LC (*22*). Nevertheless, it is possible that α_2-agonists and opiates hyperpolarize LC neurons through a common final mechanism (such as an increase in G_K). Such similarities between the effects of α_2-agonists and opiates on LC neurons may provide a basis for the proposal that clonidine suppresses symptoms of opiate withdrawal by a functionally parallel action on central noradrenergic neurons (*22, 23*).

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References and Notes

1. A. Dahlström and K. Fuxe, *Acta Physiol. Scand. Suppl.* 232 62, 1 (1965).
2. K. Starke, H. Montel, K. W. Gay, R. Merker, *Naunyn-Schmiedebergs Arch. Pharmakol.* 285, 133 (1974).
3. T. H. Svensson, B. S. Bunney, G. K. Aghajanian, *Brain Res.* 92, 291 (1975).
4. W. S. Young III and M. J. Kuhar, *Proc. Natl. Acad. Sci. U.S.A.* 77, 1696 (1980).
5. J. M. Cedarbaum and G. K. Aghajanian, *Brain Res.* 112, 413 (1976).
6. J. Marwaha and G. K. Aghajanian, *Soc. Neurosci. Abstr.* 7, 570 (1981).
7. J. M. Cedarbaum and G. K. Aghajanian, *Eur. J. Pharmacol.* 44, 375 (1977).
8. S. Z. Langer, *Biochem. Pharmacol.* 23, 1793 (1974); K. Starke, *Rev. Physiol. Biochem. Pharmacol.* 77, 1 (1977); S. Berthelsen and W. A. Pettinger, *Life Sci.* 21, 595 (1977).
9. J. Korf, B. S. Bunney, G. K. Aghajanian, *Eur. J. Pharmacol.* 25, 165 (1974); S. Nakamura, *J. Physiol. (London)* 267, 641 (1977); J. M. Cedarbaum and G. K. Aghajanian. *Life Sci.* 23, 1383 (1978); S. L. Foote, G. Aston-Jones, F. E. Bloom, *Proc. Natl. Acad. Sci. U.S.A.* 77, 3033 (1980).
10. G. K. Aghajanian, J. M. Cedarbaum, R. Y. Wang, *Brain Res.* 136, 570 (1977).
11. K. Watabe and T. Satoh, *ibid.* 165, 343 (1979).
12. W. J. Lederer, A. J. Spindler, D. A. Eisner, *Pfluegers Arch.* 381, 287 (1979).
13. R. C. Graham and M. J. Karnovsky, *J. Histochem. Cytochem.* 14, 291 (1966).
14. To increase the stability of recording, a cluster of four No. 0 insect pins (Clay Adams) (set 2 mm apart in a square pattern and held in a slotted plate) was placed vertically into the brain surrounding the recording site. The pins seemed to improve stability by damping the transmission of cardiac and pulmonary pulsations within the brain near the recording site: high-gain d-c recordings showed a dramatic decrease in pulsatile potentials after placement of the pins. Pulsations were also reduced if the membrane over the cisterna magna was punctured to allow for drainage of cerebrospinal fluid. Stability was also improved in some cases if body temperature was allowed to drop several degrees below 36°C; no effect of lowered body temperature was observed on LC cell properties. Animals were also given O_2 by nasal tube to prevent labored respiration.
15. W. C. deGroat and R. L. Volle, *J. Pharmacol. Exp. Ther.* 154, 1 (1966).
16. D. A. Brown and M. P. Caulfield, *Br. J. Pharmacol.* 65, 435 (1979).
17. L. Stjärne, *Neuroscience* 3, 1147 (1978).
18. J. P. Horn and D. A. McAfee, *J. Physiol. (London)* 301, 191 (1980).
19. K. Krnjević and A. Lisiewicz, *ibid.* 225, 363 (1972); E. Barrett and J. Barrett, *ibid.* 255, 373 (1976).
20. J. R. Hotson and D. A. Prince, *J. Neurophysiol.* 43, 409 (1980); P. A. Schwartzkroin and C. E. Stafstrom, *Science* 210, 1125 (1980).
21. C. M. Pepper and G. Henderson, *Science* 209, 394 (1980).
22. G. K. Aghajanian, *Nature (London)* 276, 186 (1978).
23. M. E. Gold, D. E. Redmond, Jr., H. D. Kleber, *Lancet* 1978-II, 599 (1978).
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Oxytocin Receptors and Human Parturition: A Dual Role for Oxytocin in the Initiation of Labor

Abstract. *The concentration of oxytocin receptors increased in the myometrium of pregnant women and reached maximum levels in early labor. Concentrations of oxytocin receptors were also high in the decidua and reached a maximum at parturition. In vitro, prostaglandin production by the decidua, but not by the myometrium, was increased by the addition of oxytocin. Oxytocin may therefore stimulate uterine contractions by acting both directly on the myometrium and indirectly on decidual prostaglandin production. Oxytocin receptors are probably crucial for the onset of human labor, and the stimulus for the increase in uterine prostaglandins may be oxytocin originating from the fetus.*

The mechanism of the initiation of human parturition remains an enigma. The concentrations of estrogen and progesterone, the main regulatory hormones in the maternal circulation, do not appear to change at the onset of parturition (*1*). Activation of the fetal adrenals, an important factor in the onset of parturition in sheep and goat, does not seem to be of critical importance for the timing of parturition in the human. Oxytocin and prostaglandins, potent stimulators of uterine contractions, are secreted during human parturition (*2*), but whether their concentrations in the maternal circulation increase as a cause or as a consequence of uterine contractions is not known; nor has a stimulus been detected for the increased production of prostaglandins during labor.

The absence in pregnant women of any of the clear and consistent changes in the concentrations of humoral factors that are associated with parturition in many animal species prompted us to search for changes at the tissue level. Soloff *et al.* (*3*) demonstrated that myometrial oxytocin receptor concentrations in pregnant rats increased shortly before parturition and reached maximum levels at delivery. Our purpose in the present study was to measure the concentration of oxytocin receptors in the uterus of pregnant women and to determine whether this concentration increases at the time of parturition. The discovery, in the course of this investigation, of high levels of oxytocin receptors in uterine decidua prompted a further study of the role of these receptors in uterine physiology.

Samples of myometrium and decidua parietalis were obtained from women delivering by cesarean sections before or at term. Samples of myometrium and endometrium were also obtained from the uteri of nonpregnant women undergoing hysterectomy. All tissues were placed on ice and transported to the laboratory for storage at −85°C until assayed. The oxytocin receptor concentrations were measured in a crude membrane fraction of myometrial and decidual homogenates (pellet sedimenting between 10,000g and 100,000g) as described (*4*), with [^{3}H]tyrosine-oxytocin being used as the radioactive ligand. The buffer used for homogenization contained 1 mM EDTA to dissociate endogenous oxytocin from its binding sites. This dissociation permitted us to determine the total number of receptor sites when the samples were exposed to endogenous oxytocin in vivo (*4*). Scatchard analyses were performed with increasing concentrations of unlabeled oxytocin. Nonspecific binding was measured by the addition of 0.2 μM unlabeled oxytocin. In many instances, a single point assay was performed in duplicate with a subsaturating concentration of [^{3}H]oxytocin (0.6 nM). This concentration of [^{3}H]oxytocin was used to minimize nonspecific binding, which was about 20

percent of the maximum amount of oxytocin bound. Because Scatchard analyses indicated that the affinity of receptor sites for oxytocin was uniform in separate portions of the uterus and throughout gestation (apparent affinity constant, K_d, was 1 nM to 2 nM) (Table 1), changes in the binding of a subsaturating concentration are proportional to the changes in the total number of oxytocin receptors. The results are therefore expressed as relative amounts of oxytocin bound per milligram of DNA.

During gestation, the myometrial receptor concentrations increased from 27.6 ± 7.9 fmole per milligram of DNA in the uteri of nonpregnant women to 171.6 ± 67.4 fmole/mg in uteri at midgestation and to 1391 ± 180 fmole per milligram of DNA at term. Maximum receptor concentrations were found in the myometrium during early labor at term: 3583 ± 857 fmole per milligram of DNA. During preterm labor, the myometrial receptor concentrations (2343 ± 316 fmole per milligram of DNA) were also higher than the concentrations in normal pregnant women at term but not in labor (Table 1). The binding affinity to oxytocin did not change during gestation (Table 1), confirming the results of Sakamoto *et al.* (*5*).

The increase in oxytocin receptor concentrations was correlated with the changes in uterine sensitivity to oxytocin. The threshold dose to produce contractions decreases from about 500 to 1000 mU in nonpregnant women to 10 to 25 mU in pregnant women at term (*6*). Before term, the uterine sensitivity to oxytocin is greater in women who later deliver prematurely than in women with normal pregnancies at the same gestational age; at delivery the uterine oxytocin sensitivity is equally high in preterm and term parturients (*7*).

The oxytocin receptor concentrations were low in samples obtained in advanced labor. We ascribe this to the fact that during labor the cervix and lower uterine segment are pulled up with the result that the incision is now made through tissue which is anatomically different from that incised before or in early labor. In samples obtained at the upper end of a longitudinal section in advanced labor, the receptor concentration was 2604 ± 143 fmole per milligram of DNA (N = 3), whereas at the lower end the concentration was 501 ± 65 fmole (N = 3), similar to the values from transverse incision in advanced labor and thus supporting our contention.

We have shown previously in rats that uterine sensitivity and the magnitude of the uterine response to oxytocin are directly related to the concentration of uterine oxytocin receptors (*8*). The increased concentration of receptors, therefore, allows for an increase in the concentration of the oxytocin-receptor complex in the face of the relatively low concentrations of circulating oxytocin e[illegible]ntered during early stages of labor [2 [illegible] 10^{-11} to $1 \times 10^{-10}M$ (*2*)]. The elicitation of hormone response with only partial receptor occupancy has been shown with a number of systems, including neurohypophyseal hormones (*9*).

An unexpected finding was a high concentration of oxytocin receptors in the parietal decidua, where the levels were maximum in preterm labor and in early term labor (Table 1). The decidual receptors had the same affinity for oxytocin as the myometrial receptors; the apparent K_d was $1.63 \pm 0.063 \times 10^{-9}M$ (N = 8). Decidua possesses high levels of prostaglandin synthetase activity (*10*), and endometrial and uterine prostaglandin synthesis is stimulated by oxytocin in sheep and rat (*11*). We considered the possibility that the oxytocin binding sites in the decidua mediate the increase in prostaglandin synthesis, and therefore measured the influence of oxytocin on the production of prostaglandins E and F by decidual and myometrial tissues in vitro. Oxytocin increased the prostaglandin production in decidua but did not influence myometrial prostaglandin production (Fig. 1). Moreover, the basal and oxytocin-stimulated prostaglandin productions in the decidua were significantly higher in samples taken after the onset of labor than before labor.

Plasma oxytocin concentrations were measured by radioimmunoassay (*12*) in

Table 1. Oxytocin receptors in samples of human uteri obtained at low flap cesarean sections (transverse incision). The values (means ± standard error) are expressed as femtomoles of oxytocin bound per milligram of DNA. Values in each vertical row with different superscripts are significantly different; Student's t-test, $P < .05$.

Group	N	Myometrium	N	K_d (nM)	N	Decidua	N	K_d (nM)
Nonpregnant, menstruating	14	$27.6^a \pm 7.97$	4	2.7 ± 0.49	6	$24.9^{a*} \pm 5.59$		
Pregnant (13 to 17 weeks)	5	$171.6^b \pm 67.4$	1	1.85	1	629^b	1	1.4
Preterm labor (28 to 36 weeks)	8	$2353^c \pm 358$	6	1.63 ± 0.22	8	$3673^c \pm 947$	4	1.75 ± 0.23
Before labor (37 to 43 weeks)	6	$1391^d \pm 180$	6	1.65 ± 0.74	6	$1510^d \pm 382$	2	2.25 ± 0.35
Early labor† (37 to 43 weeks)	5	$3468^c \pm 886$	5	1.44 ± 0.12	3	$3177^c \pm 1426$		
Advanced labor‡ (37 to 43 weeks)	7	$257^b \pm 104$	3	2.03 ± 0.7	2	$786^{b,d}$	1	2.0

*Endometrium. †Patients scheduled for cesarean section when labor began. ‡Emergency cesarean sections.

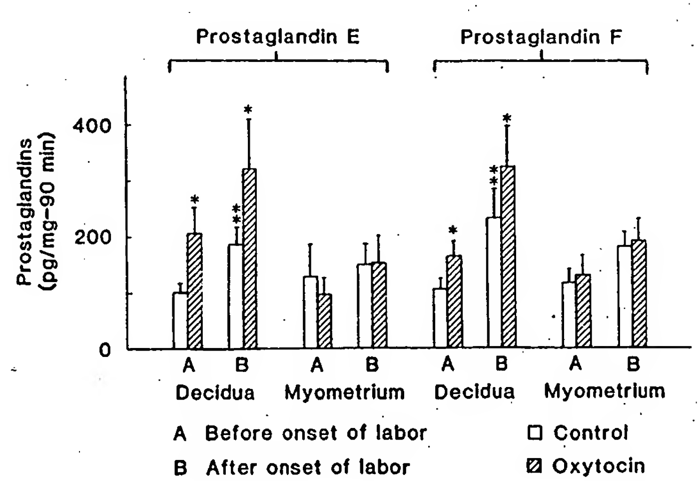

Fig. 1. Influence of oxytocin on decidual and myometrial prostaglandin production in vitro. Tissues were placed in ice-cold Krebs-Ringer solution containing a prostaglandin synthetase inhibitor (Amfenac, $1.4 \times 10^{-5}M$) and transported to the laboratory. They were then rinsed and incubated in regular Krebs-Ringer containing glucose, pH 7.4, at 35°C, under an atmosphere of 95 percent O_2 and 5 percent CO_2 with or without 10 mU of oxytocin per milliliter. After 30 minutes the medium was removed for assay and fresh medium was added. Prostaglandins were extracted from the acidified medium (pH 4.5) with a mixture of ethyl acetate and cyclohexane (1:1 by volume); portions of the dried extracts were assayed for prostaglandins E and F by means of specific radioimmunoassays (*15*).

maternal blood taken shortly before surgery and in umbilical arterial and venous blood taken at delivery (five to eight samples were obtained for each group). Before the onset of labor, levels were low in maternal (27.6 ± 10 pg/ml) and umbilical arterial blood (11.8 ± 6.2 pg/ml). In very early labor, the maternal level was unchanged (22.7 ± 6.3 pg/ml) but the umbilical arterial level significantly elevated (36.1 ± 11.7 pg/ml). In advanced labor both maternal (45.2 ± 17 pg/ml) and umbilical arterial oxytocin levels (57.3 ± 16 pg/ml) were higher than before labor.

From these results we propose that the following sequence of events results in the initiation of labor. The concentration of oxytocin receptors increases dramatically during gestation, probably under the influence of the rising estrogen levels. Evidence for the stimulation of oxytocin receptor formation by estrogens has been found in rabbits (*13*) and rats (*4*, *8*). Near term, the rapid fetal growth rate accelerates uterine distension which probably contributes to the increase in oxytocin receptors toward term, as shown in rats (*14*). Although there is no dramatic increase in circulating oxytocin at the onset of labor, the increasing concentration of receptors lowers the oxytocin threshold to the point where activation of the myometrium occurs. Simultaneously, oxytocin binds to the receptors in the decidua, stimulating prostaglandin synthesis. The released prostaglandins diffuse into the adjacent myometrium and enhance the oxytocin-induced contractions. We have shown that oxytocin-induced contractions will not dilate the cervix and lead to progressive labor unless there is simultaneous prostaglandin release (*15*). The coupling of the oxytocin receptor activation and prostaglandin synthetase activity in the decidua therefore appears to be a crucial event in the initiation of labor. Additional support for this concept is provided by the fact that both ethanol, which inhibits oxytocin release, and prostaglandin synthetase inhibitors like indomethacin, inhibit labor contractions and can be used to prevent preterm birth (*16*).

We have confirmed the fetal secretion of oxytocin at term and the arteriovenous difference in oxytocin concentrations in the umbilical cord. Although experimental limitations make it difficult to document a transfer of fetal oxytocin through the human placenta and fetal membranes, such transfer has been shown in guinea pig and baboon (*17*). The amniotic fluid at term contains considerable amounts of oxytocin (*12*), and morphological studies of the human fetal membranes at term support the view that the circulating amniotic fluid, after traversing the amnion, will continue through the intercellular canaliculi of the chorionic cytotrophoblast to reach the decidua parietalis (*18*). We therefore postulate that fetal oxytocin can provide a stimulus for the increased production of prostaglandins at the onset of labor.

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References and Notes

1. F. Fuchs and A. Klopper, Eds., *Endocrinology of Pregnancy* (Harper & Row, New York, 1977), pp. 76–122.
2. K. Green, M. Bygdeman, M. Toppozada, N. Wiqvist, *Am. J. Obstet. Gynecol.* **120**, 25 (1974); M. Y. Dawood, K. S. Raghavan, C. Pociask, F. Fuchs, *Obstet. Gynecol.* **51**, 2 (1978).
3. M. S. Soloff, M. Alexandrova, M. J. Fernström, *Science* **204**, 1313 (1979).
4. A. F. Pearlmutter and M. S. Soloff, *J. Biol. Chem.* **254**, 3899 (1979); M. Alexandrova and M. Soloff, *Endocrinology* **106**, 730 (1980).
5. H. Sakamoto, K. Den, K. Yamamoto, T. Arai, S. Kawai, *Endocrinol. Jpn.* **26**, 575 (1979).
6. E. M. Coutinho and A. C. V. Lopes, *Am. J. Obstet. Gynecol.* **102**, 479 (1968); E. M. Coutinho, J. Adeodata Filho, R. Zavier, A.-R. Fuchs, F. Fuchs, *J. Obstet. Gynaecol. Br. Commonw.* **77**, 164 (1970); E. Aburel, V. Petrescu, E. Radulescu, *Obstet. Ginecol.* **4**, 55 (1954).
7. K. Takahashi, F. Diamond, J. Bieniarz, H. Yen, L. Burd, *Am. J. Obstet. Gynecol.* **136**, 774 (1980).
8. M. S. Soloff, *Biochem. Biophys. Res. Commun.* **65**, 205 (1975); A.-R. Fuchs, M. S. Soloff, M. Alexandrova, S. Periyasami, paper presented at the Endocrine Society Annual Meeting, Washington, D.C., June 1980.
9. P. Eggena, L. Schwartz, R. Walter, *J. Gen. Physiol.* **56**, 250 (1970).
10. E. A. Willman and W. P. Collins, *Acta Endocrinol. (Copenhagen)* **87**, 632 (1978); M. D. Mitchell, J. Bibby, B. R. Hicks, A. C. Turnbull, *Prostaglandins* **5**, 377 (1978); T. Okazaki *et al.*, *Am. J. Obstet. Gynecol.* **139**, 373 (1981).
11. J. S. Roberts, J. A. McCracken, V. Gavagan, M. S. Soloff, *Endocrinology* **99**, 1107 (1976); W. Chan, *Biol. Reprod.* **17**, 548 (1977); G. A. Campos, G. C. Liggins, R. F. Seamark, *Prostaglandins* **20**, 297 (1980).
12. M. Y. Dawood, O. Ylikorkala, D. Trivedi, F. Fuchs, *J. Clin. Endocrinol. Metab.* **49**, 429 (1979).
13. R. Nissenson, G. Flouret, O. Hechter, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2044 (1978).
14. A.-R. Fuchs, S. Periyasami, M. S. Soloff, paper presented at the Annual Meeting of the Society for Gynecologic Investigation, St. Louis, March 1981.
15. P. Husslein, A.-R. Fuchs, F. Fuchs, *Am. J. Obstet. Gynecol.* **141**, 688 (1981); A.-R. Fuchs, P. Husslein, F. Fuchs, *ibid.* p. 694.
16. F. Fuchs, A.-R. Fuchs, V. T. Poblete, A. Risk, *ibid.* **99**, 627 (1967); H. Zuckerman, V. Reiss, I. Rubenstein, *Obstet. Gynecol.* **44**, 787 (1974).
17. A. M. Burton, D. V. Illingworth, J. R. G. Challis, *J. Endocrinol.* **60**, 499 (1974); M. Y. Dawood, N. H. Lauersen, D. Trivedi, O. Ylikorkala, F. Fuchs, *Acta Endocrinol. (Copenhagen)* **91**, 704 (1979).
18. H.-N. Minh, D. Doubin, A. Smadja, L. Orcel, *Eur. J. Obstet. Gynecol. Reprod. Biol.* **10**, 213 (1980).

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Rapid Electronic Autofluorography of Labeled Macromolecules on Two-Dimensional Gels

Abstract. *The feasibility of electronically locating and measuring tritium-labeled macromolecules directly on dried electrophoretic gels has been demonstrated. This new procedure eliminates the usual long film exposure in autofluorography and the attendant delay in processing and data reduction. An image intensifier and electronic camera tube are used to integrate the light produced by the tritium interaction with a scintillator incorporated in the gel. Preliminary results show that, compared to film, the exposure is reduced 100 to 1000 times. The response to low activity levels is improved, and spatial resolution is maintained. A proposed instrument could be used for measuring other isotopes as well as fluorescent and visible stains.*

Investigators in many biological research laboratories are examining normal and abnormal human and animal proteins with one- and two-dimensional separation techniques to produce protein maps of plasma, urine, and other materials. Projects are under way to compile a complete protein index of humans (*1*). In other laboratories recombinant DNA techniques are being used to study the basic properties of DNA and to manipulate DNA fragments so as to "engineer" organisms that can manufacture scarce substances such as insulin, growth hormone, and interferon (*2*). Still other researchers are seeking to distinguish tissues, particularly tumors, or to detect genetic diseases (*3*). Essential to much of this research and development are the sequence analysis of DNA fragments and the screening of clones for specific genes. Both techniques require the detection of radioactive nucleic acid or antibody by autoradiography (*4*).

In the projects and techniques mentioned above, autoradiography or autofluorography is used in which x-ray film is exposed for hours or days in order to visualize the distribution and the amounts of labeled macromolecules. The

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Rosiglitazone (BRL49653), a PPARγ-selective agonist, causes peroxisome proliferator-like liver effects in obese mice

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Abstract The PPAR (peroxisome proliferator activated receptor) transcription factors are ligand-activated nuclear receptors that regulate genes involved in lipid metabolism and homeostasis. PPARα is preferentially expressed in liver and PPARγ preferentially in adipose tissue. Activation of PPARα leads to peroxisome proliferation and increased β-oxidation of fatty acids in rodents. PPARγ-activation leads to adipocyte differentiation and improved insulin signaling of mature adipocytes. Both PPAR receptors are believed to be functional targets for treatment of hyperlipidemia in man. We have treated obese diabetic mice (*ob/ob*), which have highly elevated levels of plasma triglycerides, glucose and insulin, for 1 week with WY14,643 (180 μmol/kg/day), a selective PPARα agonist, or rosiglitazone (BRL49653; 2.5 μmol/kg/day), a selective PPARγ agonist. The doses used produce a similar therapeutic effect in both treatment groups (lowering of triglycerides and glucose). High resolution two-dimensional gel electrophoresis of livers showed that WY14,643 and rosiglitazone both produced changes in expression pattern of many proteins involved in peroxisomal fatty acid β-oxidation. However, similar experiments performed in lean mice showed significant up-regulation of these proteins only with WY14,643 treatment. Furthermore, the proteins up-regulated by the drugs in obese mice had a higher basal expression in obese controls compared to the lean littermates. Liver PPARγ mRNA levels were determined and we observed that PPARγ2 mRNA levels were elevated in obese mice compared to lean littermates. As PPARα and PPARγ recognize similar DNA response elements, it is likely that the effects of rosiglitazone on PPARα responsive genes in livers of the *ob/ob* mice are mediated by PPARγ2.—Edvardsson, U., M. Bergström, M. Alexandersson, K. Bamberg, B. Ljung, and B. Dahllöf. Rosiglitazone (BRL49653), a PPARγ-selective agonist, causes peroxisome proliferator-like liver effects in obese mice. *J. Lipid Res.* 1999. 40: 1177–1184.

Supplementary key words BRL49653 • WY14,643 • peroxisome proliferation • PPAR • proteomics • *ob/ob* • obese mice • insulin resistance • two-dimensional gel electrophoresis

Peroxisome proliferator activated receptors (PPAR) are nuclear transcription factors that heterodimerize with RXRα and activate a multitude of genes involved in lipid metabolism (1–3). There are three PPAR subtypes known to date with different tissue distribution; PPARα is highly expressed in liver and kidney and PPARδ is ubiquitously expressed. The PPARγ protein exists in two isoforms, γ1 and γ2. Profiling their relative abundance revealed that PPARγ1 is expressed in several tissues, whereas PPARγ2 is preferentially expressed in adipose tissue (1, 3–6). The γ1 and γ2 isoforms arise from differential promoter usage, which results in 30 additional N-terminal amino acids in PPARγ2. Also, a third PPARγ mRNA transcript has recently been reported (7). This transcript gives rise to a protein identical to PPARγ1 and is preferentially expressed in adipose tissue and large intestine. It has been shown that fatty acids and/or fatty acid metabolites are activators of PPARs (8–12) and it is possible to view PPARs as physiological sensors of intracellular lipid/fatty acid concentrations (1, 2, 10).

In rodent livers, activation of PPARα promotes increased fatty acid oxidation in peroxisomes, mitochondria and microsomes, and induces proliferation of peroxisomes (4, 13–15). Several proteins involved in lipid metabolism, such as lipoprotein lipase, apolipoproteins A-I, A-II and C-III, and cytochrome P450 4A, are regulated by PPARα (1, 15, 16). Ligand activation of PPARγ leads to adipocyte differentiation in cell culture as well as in whole animals (3, 17–22). In white adipose tissue, expression of adipocyte fatty acid binding protein (aFABP) (18) and expression of insulin receptors increase upon PPARγ activation (21, 23). Other proteins, such as TNFα, TNFα receptor (24), and leptin (25–27), are down-regulated in

Abbreviations: 3KCT, 3-ketoacyl CoA thiolase; ACO, acyl CoA oxidase; CYP4A, cytochrome P450-4A; aFABP, adipocyte fatty acid binding protein; PBE, peroxisomal bifunctional enzyme; PPAR, peroxisome proliferator activated receptor; PPRE, peroxisome proliferator response element; TZD, thiazolidinedione.

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adipocytes by PPARγ-activation. Taken together, activation of either PPARα or PPARγ may act to withdraw lipids and fatty acids from the circulation through increased oxidation or storage, respectively (1, 2).

It is therefore adequate that both PPARα and PPARγ may represent functional targets for hypolipidemic agents. Thus, fibrates, used for clinical treatment of severe hypertriglyceridemia, have been found to activate PPARα (4, 13) and in humans, a major effect is thought to be down-regulation of apolipoprotein C-III (2, 28, 29), an inhibitor of peripheral lipolysis. Peroxisome proliferation, as a result of PPARα activation, is considered a rodent-specific phenomenon and has not been convincingly detected in humans (30). Thiazolidinediones (TZD) (23, 31, 32) are a new class of insulin sensitizers and antidiabetic agents, with substantial effects on circulating lipids in diabetic rodents, and PPARγ has been shown to be a molecular target of TZD action (9, 33). The first TZD in clinical use is troglitazone, which recently was registered in the US for treatment of non-insulin-dependent diabetes mellitus (34). Rosiglitazone (BRL49653), a more potent PPARγ-activator, is currently near registration (35).

Reporter gene assays in transfected cell lines have shown that TZD are highly selective PPARγ-agonists (3, 12, 33). Thus, primary effects of TZD are expected in tissues expressing PPARγ. Previously, we have used proteomics (high-resolution two-dimensional electrophoresis and mass spectrometry) to characterize the effect of the peroxisome proliferator and PPARα agonist WY14,643 on the livers of obese mice (36). These experiments showed that WY14,643 induced expression of most enzymes involved in the peroxisomal fatty acid β-oxidation. Now, we have characterized the effects of both rosiglitazone and WY14,643 on the same set of proteins in the livers of both obese and lean mice. We found that whereas WY14,643 produced a similar response in both strains, rosiglitazone showed peroxisome proliferator-like effects only in obese mice. When compared to lean littermates, expression analysis of mRNA showed that obese mice have elevated liver PPARγ2 levels. We conclude that obesity in this model leads to an up-regulation in the liver of the PPARγ2 isoform and suggest that rosiglitazone causes peroxisome proliferator-like effects by activating liver PPARγ2 in obese mice.

MATERIALS AND METHODS

Materials

The IsoDalt system was from Hoefer (San Francisco, CA). Equipment for isoelectric focusing (IEF), IPGphor, Immobiline DryStrips (18 cm, pH 3-10 NL (non-linear)), IPG-buffer 3-10 NL, and Drystrip cover fluid were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Iodoacetamide and sodium thiosulfate were from Sigma (St. Louis, MO). Silver nitrate, formaldehyde, and sodium carbonate were from Merck (Darmstadt, Germany). Nonidet P-40 (NP-40) was from United States Biochemical Corp. (Cleveland, OH). Duracryl (30%, 0.65% Bis) and all other chemicals were electrophoresis grade, obtained from ESA Inc. (Chelmsford, MA). WY14,643 was purchased from Sigma (St. Louis, MO) and rosiglitazone was obtained from Medicinal Chemistry, Astra Hässle AB.

Animals and drug treatment

Obese mice (*ob/ob*; Umeå) and their lean littermates (+/?) were from Bomholtgård Breeding and Research Centre, Denmark (genotyping of lean mice was not considered critical for this study and for practical reasons we therefore used +/? lean control mice). The 7-week-old animals were treated orally once daily for 1 week with WY14,643 (180 μmol/kg/day) or rosiglitazone (2.5 μmol/kg/day). On the last day of the experiment the mice were anesthetized in CO_2 and exsanguinated via a carotid artery. The blood was collected in vials containing EDTA.

Metabolic effects

In plasma, insulin was analyzed using a rat insulin RIA kit (RI-13K; Linco, St. Louis, MO) and triglycerides (TG) and glucose were analyzed spectrophotometrically on the Cobas Mira plus (Hoffman la Roche, Basel, CH) using Calibrator Human (07 3718 6; Roche, Basel, CH) as calibrant. For TG the enzymatic kit "Triglycerides/Glycerol Blanking" (450032; Boehringer Mannheim, Indianapolis, IN) was used, and for glucose "Glucose HK" (07 3672 4; Roche, Basel, CH).

Sample preparation for two-dimensional electrophoresis

The apical ends of the left liver lobes were rapidly removed, frozen in liquid nitrogen, and stored at −150°C until subsequent electrophoretic analysis. Liver samples were weighed and homogenized with a glass/Teflon homogenizer (5 strokes at 400 rpm) in 8 volumes of solubilizing solution (8 M Urea, 0.3% (w/v) dithiothreitol (DTT), 2% (v/v) NP-40, and 2% (v/v) IPG-buffer 3-10 NL). To remove solid tissue, the homogenate was centrifuged at 100 000 *g* for 30 min at 15°C. The supernatant was carefully removed and immediately frozen at −70°C.

First dimension (isoelectric focusing)

Electrophoresis of mouse liver proteins was performed on individual samples, four from each treatment group and from lean controls, and six from obese controls. Immobiline DryStrips (18 cm, pH 3-10 NL (non-linear)) were used for isoelectric focusing. Each strip was rehydrated for 12 h in 350 μl of rehydration solution containing 4 μl (~100 μg) of solubilized liver protein. The rehydration solution consisted of 8 M urea, 2% (v/v) NP-40, 0.3% (w/v) DTT, and 0.5% (v/v) IPG-buffer 3-10 NL. The strips were run under a layer of Drystrip cover fluid, at 20°C, in a IPGphor unit according to the manufacturer's instructions. The focusing was carried out at 500 V; 1 h, 1000 V; 1 h and 8000 V; 9 h to reach a total of 74 kVh.

Equilibration of IPG gel strips

After IEF the strips were equilibrated 2 × 15 min with gentle shaking (37). The first equilibration solution contained 30% glycerol (w/v), 6 M urea, 2% SDS, 50 mM Tris/HCl, pH 8.8, 65 mM DTT, and a trace of bromophenol blue as tracking dye. The second equilibration was carried out in same solution, except that DTT was replaced by 260 mM iodoacetamide.

Second dimension (SDS-PAGE)

The gels used in this study were continuous 14% T, 0.3% C gels in the format of 23 × 20 × 0.1 cm. After the equilibration, each IEF strip was drained on a filter paper and immersed in SDS running buffer (24 mM Tris base, 0.2 M glycine, and 0.1% SDS) before it was sealed at the top of the second dimension gel with 1% agarose in SDS running buffer. Electrophoresis was performed in the IsoDalt tank (Hoefer) at 100 V for ~19 h, until the tracking dye reached the anodic end of the gels.

Silver staining

Analytical gels were silver stained according to Shevchenko et al. (38) with some modifications. The gels were fixed in 50% ethanol, 5% acetic acid for 1 h, washed in 50% ethanol for 30 min and additionally 60 min with water to remove the remaining acid. Thereafter, the gels were sensitized by a 1-min incubation in 0.02% sodium thiosulfate and rinsed with two changes of distilled water for 1 min each. After rinsing, the gels were incubated in 0.1% silver nitrate for 30 min. After incubation, the gels were rinsed twice with distilled water for 1 min and then developed in 0.04% formaldehyde, 2% sodium carbonate. When the developer turned yellow (~30 sec) it was discarded and replaced with fresh solution. When desired intensity of staining was achieved (~3.5 min), the development was terminated by discarding the reagent, followed by washing with 5% acetic acid for 5–10 min. Finally, the gels were washed in water and stored in sealed plastic bags at 4°C.

Image analysis

Silver-stained gels were imaged using a cooled CCD camera-based instrument, Fluor-S™ MultiImager (Bio-Rad). Raw scans were processed by the 2D software PDQuest (PC version 5.1) according to the following procedure: make gel image, ball background subtraction (radius 80), two times averaging smooth, spots detected and fitted to Gaussian volumes (39). After manual landmarking of approximately 140 spots in each gel image, the spot patterns of the different gels were automatically matched to each other and each spot was given a unique identification number (SSP). Individual quantifications of resolved proteins were normalized to the total intensity of detected spots in each gel. To find spots that differed quantitatively between the control and treated groups, the average intensities (n = 4–6) of resolved spots were compared using the statistical and quantitative functions within the PDQuest software.

RNA isolation

Fresh liver samples were rinsed in PBS and frozen immediately in liquid N_2. Total RNA was prepared using RNA-Stat 60 (Tel-Test, Inc., Friendswood, TX) according to the manufacturer's suggestions. Concentration and yield were determined by optical density measurement at 260 nm and the integrity of the RNA was verified by agarose gel electrophoresis and quantification of the 28S and 18S ribosomal bands.

Ribonuclease protection assay (RPA)

Nucleotides 1–323 (position 1 is the A in the initial ATG) from mouse PPARγ2 in a pBluescript vector (Stratagene, La Jolla, CA) served as template for PPARγ probe synthesis. ^{32}P-labeled antisense probe was generated using Promega in vitro transcription kit and the probe was purified by PAGE.

The RPAII kit (Ambion Inc., Austin, TX) was used for mRNA quantification. Briefly, 20 μg total liver RNA or 5 μg total RNA from white adipose tissue was hybridized at 45°C overnight to 80,000 cpm probe. The samples were subsequently treated with an RNaseT1/RNaseA mix at 37°C for 30 min to digest single-stranded RNA prior to separation on a 6% denaturing polyacrylamide gel. Radioactivity on the dried gel was visualized using a STORM™ phosphorimager (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Drug treatment and therapeutic effects

Male obese and lean mice were treated for 1 week with WY14,643 (180 μmol/kg/day) or rosiglitazone (2.5 μmol/kg/day), doses that were previously determined by dose–response experiments to be equally effective in male obese mice regarding lowering of plasma triglycerides and glucose (data not shown). In **Fig. 1** we show that both substances again caused similar reductions in plasma triglycerides and glucose in obese insulin-resistant mice, to levels of the lean control mice. In lean insulin-sensitive mice, drug treatment did not significantly affect triglyceride or glucose levels.

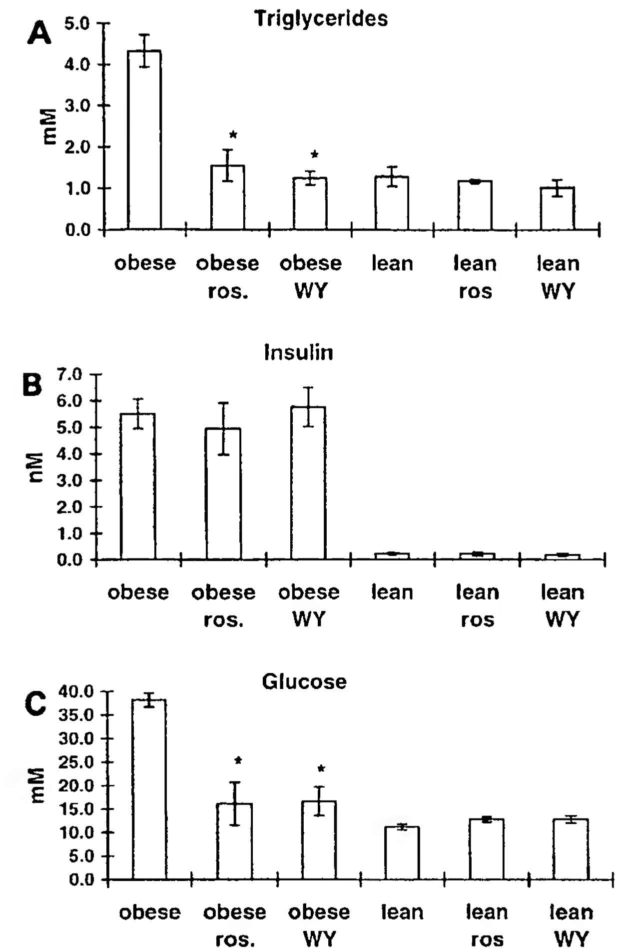


Fig. 1. Therapeutic effects of rosiglitazone and WY14,643. Plasma levels of triglycerides (A), insulin (B), and glucose (C) in obese and lean mice after 1 week treatment with rosiglitazone (2.5 μmol/kg/day) or WY14,643 (180 μmol/kg/day) in comparison to untreated obese and lean animals. Values are mean ± SE, n = 4 (obese controls, n = 6). * $P < 0.05$ using Student's t-test for each treated group versus obese or lean controls.

Two-dimensional gel electrophoresis

Liver samples, 4 from each treatment group and 6 from obese controls, were homogenized in rehydration buffer and analyzed by two-dimensional electrophoresis as described in Materials and Methods. The images of the 26 gels were captured and analyzed by using the PDQuest software as described in Materials and Methods. Previously, we have characterized the dominant response to WY14,643 in obese mice and found that 16

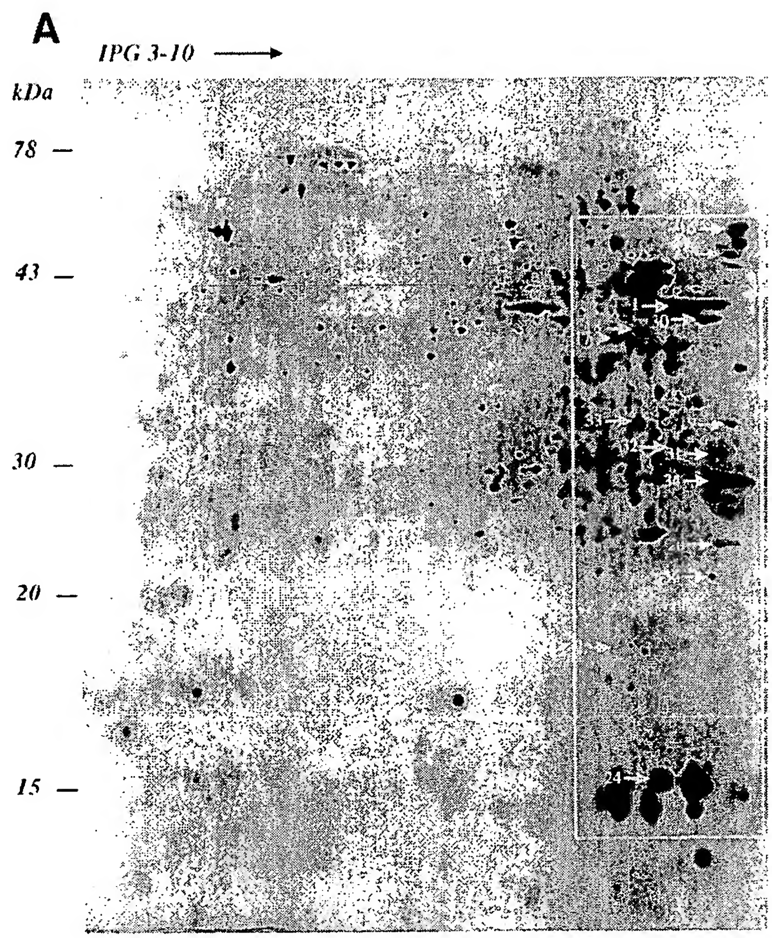
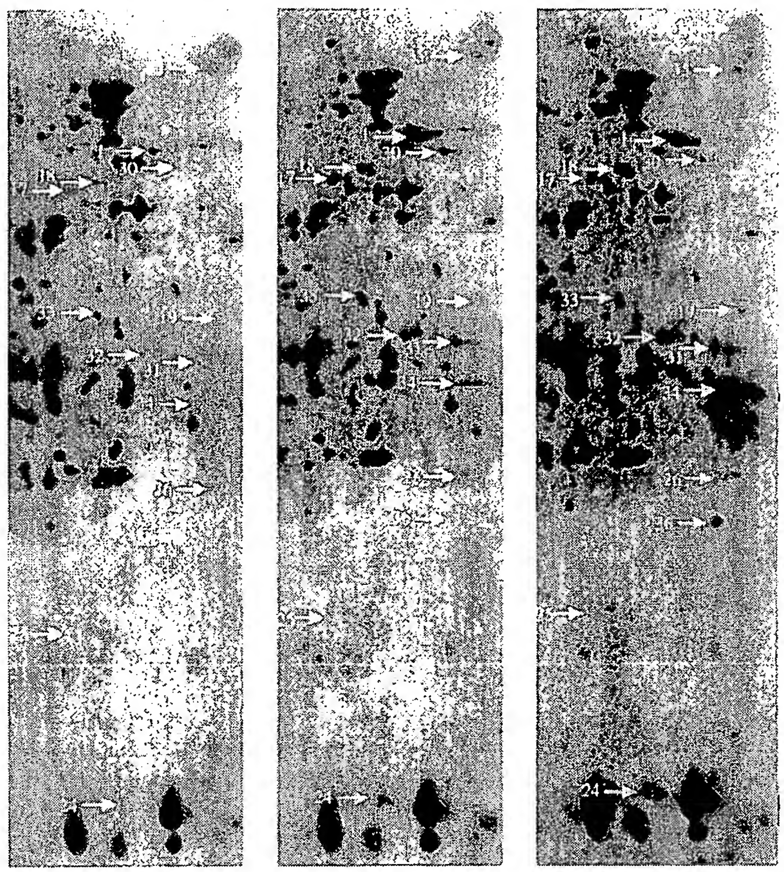


Fig. 2

spots were clearly up-regulated by the treatment. These spots were identified by in-gel tryptic digestion and mass spectrometry (MALDI-TOF and MS/MS) as described (36). Of these spots, 14 were identified as polypeptides from acyl CoA oxidase (ACO), peroxisomal bifunctional enzyme (PBE), and 3-ketoacyl thiolase (3KCT), enzymes which are central components of the peroxisomal fatty acid β-oxidation. Furthermore, one spot was identified as adipocyte fatty acid binding protein, aFABP, which may be viewed as a substrate carrier for this metabolic pathway, and one spot was identified as HMG-CoA synthase which is not directly involved in peroxisomal fatty acid β-oxidation. In this study, we focused on the regulation of the 15 spots that are components of, or are related to (aFABP), peroxisomal fatty acid β-oxidation. These spots are located in the basic region of the gels covering a pI range from 3 to 10 (see the master gel in **Fig. 2A**). Representative gels of liver samples from untreated, rosiglitazone- and WY14,643-treated obese mice are shown as cut-outs from 2D-gels in Fig. 2B. By visual inspection of the gels, we found that both rosiglitazone and WY14,643 apparently up-regulated most of these 15 spots.

Thereafter, semiquantitative data obtained from computer-aided image analysis were analyzed and we found that in obese animals, out of these 15 spots, WY14,643 and rosiglitazone induced significant up-regulation of 14 and 9 spots, respectively (Fig. 3A). As each enzyme is represented by several spots, a common finding in two-dimensional electrophoresis most likely representing cleavage and degradation occurring in vivo, it is important to note that rosiglitazone did affect at least one spot from all of the three identified peroxisomal enzymes. To explain that several spots, originating from one protein, are not regulated in a coordinated manner by pharmacological treatments would require complete knowledge of the primary sequence of each spot, as well as an understanding of each activation/degradation pathway. Such an analysis has not been possible to perform in this study. In lean mice, rosiglitazone did not cause any significant changes whereas WY14,643 caused significant up-regulation of 15 spots (Fig. 3B). It is interesting to note that all spots representing ACO, PBE, and 3KCT had a higher basal expression in obese compared to lean mice (Fig. 3C), but this was not the case for aFABP. Thus, rosiglitazone caused a similar, but somewhat less pronounced, induction of peroxisomal enzymes in obese mice as compared to WY14,643. In

B



control rosiglitazone WY14,643

Fig. 2. (A) Two-dimensional master gel with the 15 spots characterizing the major peroxisome proliferator-like response in obese mice: (1) 3-ketoacyl-CoA thiolase, (16) peroxisomal bifunctional enzyme, (17) 3-ketoacyl-CoA thiolase, (18) 3-ketoacyl-CoA thiolase, (19) peroxisomal bifunctional enzyme, (24) aFABP, (25) acyl CoA oxidase, (26) acyl CoA oxidase, (30) 3-ketoacyl-CoA thiolase, (31) peroxisomal bifunctional enzyme, (32) 3-ketoacyl-CoA thiolase, (33) acyl CoA oxidase, (34) peroxisomal bifunctional enzyme, (35) peroxisomal bifunctional enzyme, (36) peroxisomal bifunctional enzyme. (B) Enlarged sections, cut-out as indicated by the box in (A), from representative gels of liver samples from obese control, rosiglitazone- and WY14,643-treated mice. Arrows indicate spots up-regulated by the rosiglitazone and/or the WY14,643 treatment.

contrast, only WY14;643 induced this metabolic pathway in lean mice.

Liver expression of PPARγ mRNA

PPARα and PPARγ show similar binding to the peroxisomal proliferator response element (PPRE) of ACO (20). It is therefore conceivable that PPARγ, if expressed in liver, will bind to the same PPREs as PPARα and hence mimic PPARα-mediated gene regulation. Therefore, we decided to compare mRNA expression levels of PPARγ1 and PPARγ2 in livers from the lean and obese mice. As can be seen in **Fig. 4**, lean mice expressed only low levels of PPARγ1 and barely detectable amounts of PPARγ2. However, obese animals showed markedly elevated levels of the PPARγ2 isoform, whereas the PPARγ1 levels remained low. Thus, the peroxisome proliferator-like liver effects of rosiglitazone in obese mice may be due to activation of PPARγ2.

DISCUSSION

In our efforts to map the mechanisms of action of antihyperlipidemic drugs, we first characterized the response of the selective PPARα activator WY14,643 in livers from obese mice (36). We found the expected induction of central components of the peroxisomal fatty acid β-oxidation pathway, a response typical for peroxisome proliferators. Now, we asked whether the TZD rosiglitazone, reported to be a selective PPARγ-agonist (9, 33), may cause similar effects as WY14,643 in lean and obese mice.

The results are summarized as follows. *i*) At equally

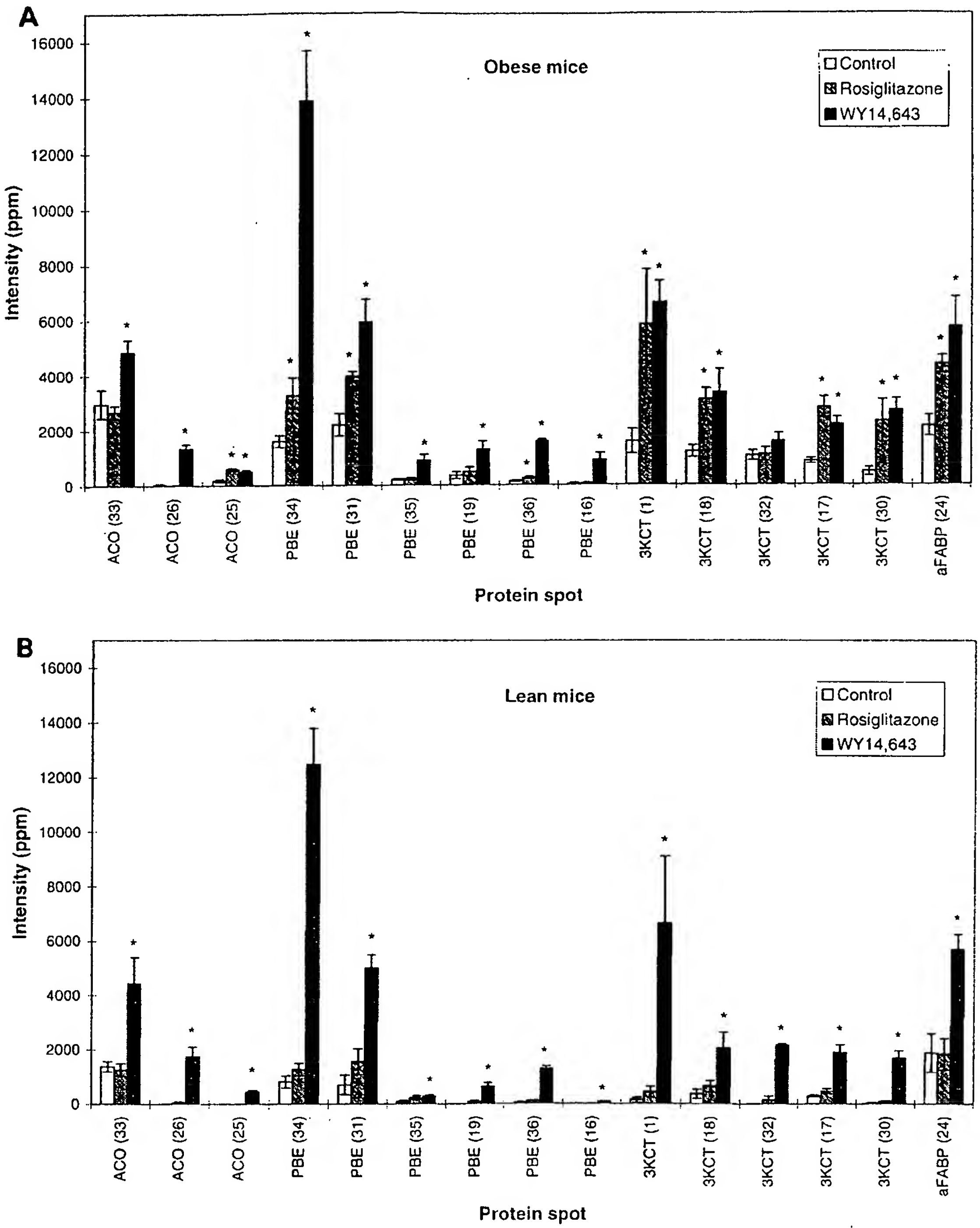


Fig. 3

effective doses in obese mice (with regard to correction of hypertriglyceridemia and hyperglycemia), rosiglitazone and WY14,643 induce similar responses identified as up-regulation of peroxisomal fatty acid β-oxidation. *ii)* In lean mice, WY14,643 exerted effects similar to those in obese mice whereas rosiglitazone had no effect on the studied proteins. *iii)* Livers from obese mice contain more PPARγ2 mRNA than livers from lean mice. To conclude, our findings suggest that the elevated levels of liver PPARγ2 in obese mice may render these animals susceptible to a peroxisome proliferator-like activation by rosiglitazone. In relation to this, it is interesting to note that others have found elevated PPARγ expression in livers from obese mice fed a high fat-containing diet (40). Also, a recent report on PPARα knockout mice, which become obese, showed an increased expression of liver PPARγ in fat-filled liver cells from males, whereas the expression of aFABP, adipoQ, and hormone-sensitive

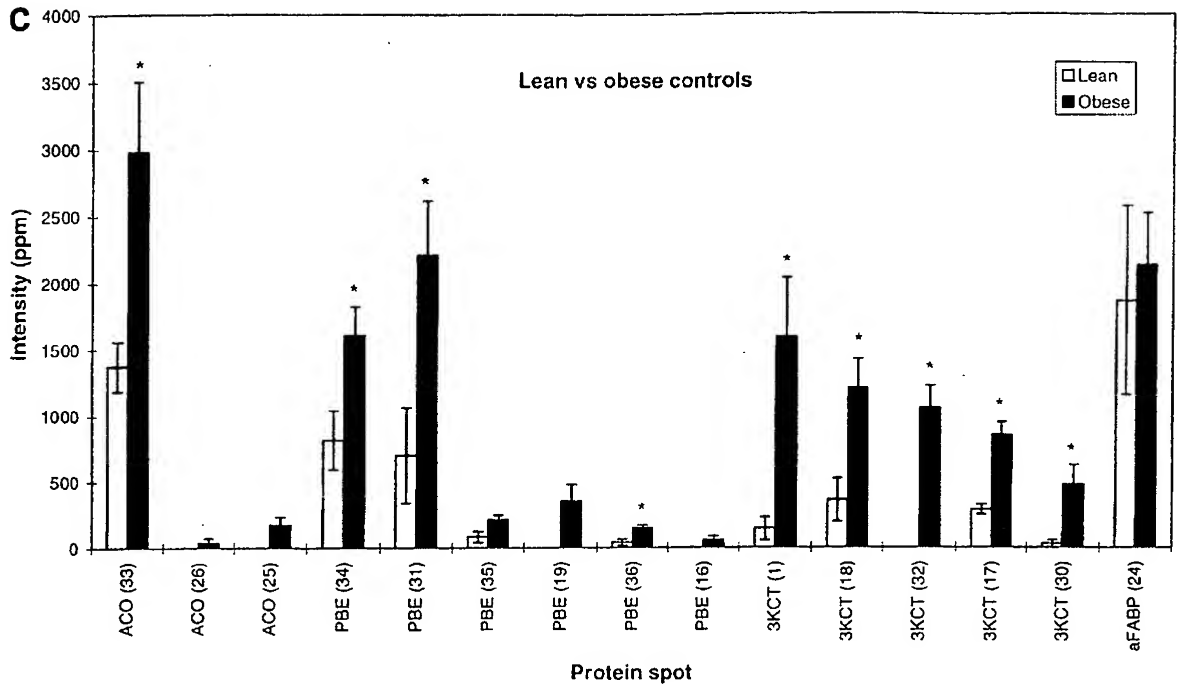


Fig. 3. A, B: Semi-quantitative data of 15 spots involved in peroxisomal fatty acid β-oxidation up-regulated by rosiglitazone (2.5 μmol/kg/day, hatched bars) or WY14,643 (180 μmol/kg/day; black bars) compared to control (white bars), (A: obese mice; B: lean mice). C: Comparison of the expression levels of the same proteins between untreated lean (white bars) and obese mice (black bars). The ordinate represents numbers given by the PDQuest software (ppm; normalized to the total intensity of all detected spots on each gel). ACO, acyl CoA oxidase; PBE, peroxisomal bifunctional enzyme; 3KCT, 3-ketoacyl thiolase; aFABP, adipocyte fatty acid binding protein. Numbers in brackets refer to corresponding numbers on the master gel in Fig. 2A. Values are mean ± SE, n = 4 (obese controls, n = 6). * $P < 0.05$ using Student's t-test for each treated group versus obese (A) or lean controls (B), and obese versus lean (C).

lipase remained unchanged (41). The results are in line with our data concerning the hepatic expression of PPARγ and aFABP in obesity. Thus, obesity and high-fat feeding regulate PPARγ levels in mice, and this may determine the response to PPAR activators in vivo. In particular, the PPARγ2 isoform may be the one that is regulated in obesity. To understand the pharmacodynamic effects of PPAR activators in humans, it will be important to investigate possible regulatory effects of obesity on hepatic PPARγ expression in human subjects.

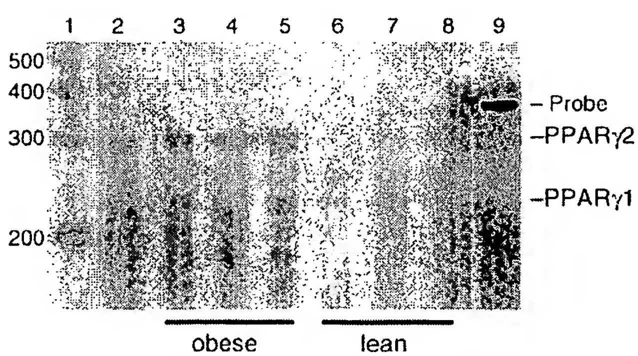


Fig. 4. PPARγ1 and PPARγ2 expression in lean and obese mice quantified by RPA. Five μg total white adipose tissue RNA from obese mice (lane 2) or 20 μg total liver RNA from 3 obese (lanes 3–5) or 3 lean (lanes 6–8) animals was hybridized to a ^{32}P-labeled antisense probe, digested with a RNaseT1/RNaseA mix, and separated on a 6% denaturing polyacrylamide gel. The probe contained the nucleotides 1–323, counting from the initiating ATG in PPARγ2, plus vector sequence to account for a total of 379 nt (lane 9, undigested probe). PPARγ1 mRNA protects a 233 nt fragment, PPARγ2 protects a 323 nt fragment. Lane 1 contains a molecular size marker.

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REFERENCES

1. Wahli, W., O. Braissant, and B. Desvergne. 1995. Peroxisome proliferator activated receptors–transcriptional regulators of adipogenesis, lipid metabolism and more. *Chem Biol.* 2: 261–266.
2. Schoonjans, K., B. Staels, and J. Auwerx. 1996. Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression. *J. Lipid Res.* 37: 907–925.
3. Spiegelman, B. M. 1998. PPAR-gamma—adipogenic regulator and thiazolidinedione receptor. *Diabetes.* 47: 507–514.
4. Issemann, I., and S. Green. 1990. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature.* **347:** 645–650.
5. Tontonoz, P., E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegel-

man. 1994. mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. *Genes & Dev.* **8**: 1224 - 1234.
6. Braissant, O., F. Foufelle, C. Scotto, M. Dauca, and W. Wahli. 1996. Differential expression of peroxisome proliferator-activated receptors (PPARs) — tissue distribution of PPAR-α, -β, and -γ in the adult rat. *Endocrinology.* **137**: 354 - 366.
7. Fajas, L., J. C. Fruchart, and J. Auwerx. 1998. PPAR-gamma-3 mRNA — a distinct PPAR-gamma mRNA subtype transcribed from an independent promoter. *FEBS Lett.* **438**: 55 - 60.
8. Yu, K., W. Bayona, C. B. Kallen, H. P. Harding, C. P. Ravera, G. McMahon, M. Brown, and M. A. Lazar. 1995. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J. Biol. Chem.* **270**: 23975 - 23983.
9. Forman, B. M., P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans. 1995. 15-Deoxy-delta(12,14)-prostaglandin J(2) is a ligand for the adipocyte determination factor PPAR-gamma. *Cell.* **83**: 803 - 812.
10. Forman, B. M., J. Chen, and R. M. Evans. 1997. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc. Natl. Acad. Sci. USA.* **94**: 4312 - 4317.
11. Devchand, P. R., H. Keller, J. M. Peters, M. Vazquez, F. J. Gonzalez, and W. Wahli. 1996. The PPAR-alpha-leukotriene B-4 pathway to inflammation control. *Nature.* **384**: 39 - 43.
12. Kliewer, S. A., J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris, and J. M. Lehmann. 1995. A prostaglandin J(2) metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. *Cell.* **83**: 813 - 819.
13. Issemann, I., R. A. Prince, J. D. Tugwood, and S. Green. 1993. The peroxisome proliferator-activated receptor:retinoid X receptor heterodimer is activated by fatty acids and fibrate hypolipidaemic drugs. *J. Mol. Endocrinol.* **11**: 37 - 47.
14. Frøyland, L., L. Madsen, H. Vaagenes, G. K. Totland, J. Auwerx, H. Kryvi, B. Staels, and R. K. Berge. 1997. Mitochondrion is the principal target for nutritional and pharmacological control of triglyceride metabolism. *J. Lipid Res.* **38**: 1851 - 1858.
15. Simpson, A. 1997. The cytochrome P450 4 (CYP4) family. *Gen. Pharmacol.* **28**: 351 - 359.
16. Schoonjans, K., J. Peinadoonsurbe, A. M. Lefebvre, R. A. Heyman, M. Briggs, S. Deeb, B. Staels, and J. Auwerx. 1996. PPAR-alpha and PPAR-gamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J.* **15**: 5336 - 5348.
17. Hiragun, A., M. Sato, and H. Mitsui. 1988. Preadipocyte differentiation in vitro: identification of a highly active adipogenic agent. *J. Cell. Physiol.* **134**: 124 - 130.
18. Kletzien, R. F., S. D. Clarke, and R. G. Ulrich. 1992. Enhancement of adipocyte differentiation by an insulin-sensitizing agent. *Mol. Pharmacol.* **41**: 393 - 398.
19. Tontonoz, P., E. D. Hu, and B. M. Spiegelman. 1994. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. *Cell.* **79**: 1147 - 1156.
20. Brun, R. P., P. Tontonoz, B. M. Forman, R. Ellis, J. Chen, R. M. Evans, and B. M. Spiegelman. 1996. Differential activation of adipogenesis by multiple PPAR isoforms. *Genes & Dev.* **10**: 974 - 984.
21. Young, P. W., M. A. Cawthorne, P. J. Coyle, J. C. Holder, G. D. Holman, I. J. Kozka, D. M. Kirkham, C. A. Lister, and S. A. Smith. 1995. Repeat treatment of obese mice with BRL 49653, a new potent insulin sensitizer, enhances insulin action in white adipocytes. Association with increased insulin binding and cell-surface GLUT4 as measured by photoaffinity labeling. *Diabetes.* **44**: 1087 - 1092.
22. Hallakou, S., L. Doare, F. Foufelle, M. Kergoat, M. Guerre-Millo, M. F. Berthault, I. Dugail, J. Morin, J. Auwerx, and P. Ferre. 1997. Pioglitazone induces in vivo adipocyte differentiation in the obese Zucker Fa/Fa rat. *Diabetes.* **46**: 1393 - 1399.
23. Chang, A. Y., B. M. Wyse, and B. J. Gilchrist. 1983. Ciglitazone, a new hypoglycemic agent. II. Effect on glucose and lipid metabolisms and insulin binding in the adipose tissue of C57BL/6J-ob/ob and -+/? mice. *Diabetes.* **32**: 839 - 845.
24. Hofmann, C., K. Lorenz, S. S. Braithwaite, J. R. Colca, B. J. Palazuk, G. S. Hotamisligil, and B. M. Spiegelman. 1994. Altered gene expression for tumor necrosis factor-α and its receptor during drug and dietary modulation of insulin resistance. *Endocrinology.* **134**: 264 - 270.
25. Macdougald, O. A., C. S. Hwang, H. Y. Fan, and M. D. Lane. 1995. Regulated expression of the obese gene product (Leptin) in white adipose tissue and 3T3-L1 adipocytes. *Proc. Natl. Acad. Sci. USA.* **92**: 9034 - 9037.
26. Zhang, B., M. P. Graziano, T. W. Doebber, M. D. Leibowitz, S. White-Carrington, D. M. Szalkowski, P. J. Hey, M. Wu, C. A. Cullinan, P. Bailey, B. Lollmann, R. Frederich, J. S. Flier, C. D. Strader, and R. G. Smith. 1996. Down-regulation of the expression of the obese gene by an antidiabetic thiazolidinedione in Zucker diabetic fatty rats and db/db mice. *J. Biol. Chem.* **271**: 9455 - 9459.
27. Rentsch, J., and M. Chiesi. 1996. Regulation of ob gene mRNA levels in cultured adipocytes. *FEBS Lett.* **379**: 55 - 59.
28. Staels, B., N. Vudac, V. A. Kosykh, R. Saladin, J. C. Fruchart, J. Dallongeville, and J. Auwerx. 1995. Fibrates downregulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme a oxidase — a potential mechanism for the hypolipidemic action of fibrates. *J. Clin. Invest.* **95**: 705 - 712.
29. Hertz, R., J. Bisharashieban, and J. Bartana. 1995. Mode of action of peroxisome proliferators as hypolipidemic drugs — suppression of apolipoprotein C-III. *J. Biol. Chem.* **270**: 13470 - 13475.
30. Ashby, J., A. Brady, C. R. Elcombe, B. M. Elliott, J. Ishmael, J. Odum, J. D. Tugwood, S. Kettle, and I. F. Purchase. 1994. Mechanistically based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum. Exp. Toxicol.* **13**: S1 - 117.
31. Colca, J. R., and D. R. Morton. 1990. Antihyperglycaemic thiazolidinediones: ciglitazone and its analogues. *In* New Antidiabetic Drugs. C. J. Bailey and P. R. Flatt, editors. Eldered Smith-Gordon, London. 255 - 261.
32. Cantello, B. C. C., M. A. Cawthorne, G. P. Cottam, P. T. Duff, D. Haigh, R. M. Kindley, C. A. Lister, S. A. Smith, and P. L. Thurlby. 1994. [[Omega-(heterocyclylamino)alkoxy]benzyl]-2, 4-thiazolidinediones as potent antihyperglycemic agents. *J. Med. Chem.* **37**: 3977 - 3985.
33. Lehmann, J. M., L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer. 1995. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR-gamma). *J. Biol. Chem.* **270**: 12953 - 12956.
34. Johnson, M. D., L. K. Campbell, and R. K. Campbell. 1998. Troglitazone — review and assessment of its role in the treatment of patients with impaired glucose tolerance and diabetes mellitus. *Ann. Pharmacother.* **32**: 337 - 348.
35. Patel, J., E. Miller, and R. Patwardhan. 1998. Rosiglitazone (BRL49653) monotherapy has significant glucose lowering effect in type 2 diabetic patients. *Diabetes.* **47**: A17.
36. Edvardsson, U., M. Alexandersson, F. Nilsson, H. Brockenhuus von Löwenhielm, A-C. Nyström, B. Ljung, and B. Dahllöf. 1999. A proteome analysis of livers from obese (*ob/ob*) mice treated with the peroxisome proliferator WY14,643. *Electrophoresis.* **20**: 935 - 942.
37. Görg, A., G. Boguth, C. Obermaier, A. Posch, and W. Weiss. 1995. Two-dimensional polyacrylamide gel electrophoresis with immobilized pH gradients in the first dimension (IPG-Dalt): the state of the art and the controversy of vertical versus horizontal systems. *Electrophoresis.* **16**: 1079 - 1086.
38. Shevchenko, A., M. Wilm, O. Vorm, and M. Mann. 1996. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal. Chem.* **68**: 850 - 858.
39. Garrels, J. I. 1989. The QUEST system for quantitative analysis of two-dimensional gels. *J. Biol. Chem.* **264**: 5269 - 5282.
40. Vidalpuig, A., M. Jimenezlinan, B. B. Lowell, A. Hamann, E. Hu, B. Spiegelman, J. S. Flier, and D. E. Moller. 1996. Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. *J. Clin. Invest.* **97**: 2553 - 2561.
41. Costet, P., C. Legendre, J. More, A. Edgar, P. Galtier, and T. Pineau. 1998. Peroxisome proliferator-activated receptor α-isoform defiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. *J. Biol. Chem.* **273**: 29577 - 29585.

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ART UNIT	**PAPER NUMBER**
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DATE MAILED: 02/23/2005

2mo. due to Provoke Advisory Action. 4/23/05. Final OA initial deadline 5/23/05. Final OA

Please find below and/or attached an Office communication concerning this application or proceeding.

RECEIVED
Date(s) Docketed: Final deadline 8/23/ Notice of Appeal 5/23/05. Final deadline for Notice of Appeal 8/23/05.
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Client: UTSG:240US
Attorney(s): MBW, MRK
Initials:

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Office Action Summary	Application No.	Applicant(s)
	10/069,744	COPLAND ET AL.
	Examiner	**Art Unit**
	Shaojia A. Jiang	1617

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on 12 November 2004.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) 2-19 is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) 2-19 is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

This Office Action is a response to Applicant's amendment and response filed on November 12, 2004 wherein claims 2-19 have been amended since claim 2 has been amended. Claims 1 and 20-21 are cancelled previously.

Currently, claims 2-19 are pending in this application.

Claims 2-19 are currently under examination on the merits.

Applicant's amendment amending claim 2, filed November 12, 2004 with respect to the rejection made under 35 U.S.C. 112 first paragraph for lack of scope of enablement for <u>any</u> oxytocin-<u>mediated</u> action of record stated in the Office Action dated May 19, 2004 has been fully considered and is found persuasive to overcome the rejection since the particular oxytocin-<u>mediated</u> action has been recited. Therefore, the said rejection is withdrawn.

Applicant's amendment amending claims 8-9, filed November 12, 2004 with respect to the rejection of 8-9 made under 35 U.S.C. 112 second paragraph for use of the indefinite recitation " the thiazolidinedione comprises troglitazone" and "the thiazolidinedione comprises pioglitazone, 8RL49653, or a compound related to troglitazone" of record stated in the Office Action dated May 19, 2004 have been fully considered and found persuasive <u>only as to these particular recitation</u>.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 15-16 are rejected under 35 U.S.C. 112, first paragraph, for scope of enablement because the specification, while being enabling for the particular and specific tocolytic agents or the particular and specific beta-mimetic, the particular and specific prostaglandin inhibitors, or the particular and specific calcium-blocking agents, such as <u>ritodrine, magnesium sulfate, indomethacin, and nifedipine</u> disclosed in the specification in co-administering with thiazolidinedion herein, does not reasonably provide enablement for any substances or compounds or agents represented by "one beta-mimetic", "at least one prostaglandin inhibitor", or " one calcium-blocking agent" recited in the claims herein, for the same reasons of record stated in the Office Action dated May 19, 2004.

These recitations, "one beta-mimetic", "at least one prostaglandin inhibitor", or " one calcium-blocking agent", are seen to be merely functional language.

The instant specification fails to provide information that would allow the skilled artisan to <u>fully</u> practice the instant invention without ***undue experimentation***. Attention is directed to *In re Wands*, 8 USPQ2d 1400 (CAFC 1988) at 1404 where the court set forth the eight factors to consider when assessing if a disclosure would have required undue experimentation. Citing *Ex parte Forman*, 230 USPQ 546 (BdApls 1986) at 547 the court recited eight factors:

(1) the nature of the invention; (2) the state of the prior art; (3) the relative skill of those in the art; (4) the predictability or unpredictability of the art; (5) the breadth of the claims; (6) the amount of direction or guidance presented; (7) the presence or absence of working examples; and (8) the quantity of experimentation necessary.

The nature of the invention: The instant invention pertains the methods for reducing oxytocin-mediated action in a mammal.

The relative skill of those in the art: The relative skill of those in the art is high.

The breadth of the claims: The instant claims are deemed very broad since the claims read on any substances or compounds or agents represented by "one beta-mimetic", "at least one prostaglandin inhibitor", or " one calcium-blocking agent" in co-administering with thiazolidinedion herein for reducing any oxytocin-mediated action in a mammal.

The amount of direction or guidance presented:

Functional language at the point of novelty, as herein employed by Applicants, is admonished in *University of California v. Eli Lilly and Co.* 43 USPQ2d 1398 (CAFC, 1997) at 1406: stating this usage does "little more than outline goal appellants hope the recited invention achieves and the problems the invention will hopefully ameliorate". The CAFC further clearly states that "[A] written description of an invention involving a chemical genus, like a description of a chemical species, requires a precise definition, such as by structure, formula, [or] chemical name, of the claimed subject matter sufficient to distinguish it from other materials" at 1405(emphasis added), and that "It does not define any structural features commonly possessed by members of the genus that distinguish from others. One skilled in the art therefore cannot, as one can do with a

fully described genus, visualize or recognize the identity of the members of the genus. A definition by function, as we have previously indicated, does not suffice to define the genus.." at 1406 (emphases added).

In the instant case, "one beta-mimetic", "at least one prostaglandin inhibitor", or " one calcium-blocking agent", recited in the instant claims are purely functional distinction. Hence, these functional recitations read on any compounds that might have the recited functions. However, the specification merely provides those particular and specific compounds for each kind of functional compounds for the method of treatment herein.

Thus, Applicants functional language at the points of novelty fails to meet the requirements set forth under 35 U.S.C. 112, first paragraph. Claims employing functional language at the exact point of novelty, such as Applicants', neither provide those elements required to practice the inventions, nor "inform the public during the life of the patent of the limited of monopoly asserted" (*General Electric Company v. Wabash Appliance Corporation et al.* 37 USPQ at 468 (US Supreme Court 1938)).

The predictability or unpredictability: the instant claimed invention is highly *unpredictable* as discussed below:

It is noted that the pharmaceutical art is unpredictable, requiring each embodiment to be individually assessed for physiological activity. *In re Fisher*, 427 F.2d 833, 166 USPQ 18 (CCPA 1970) indicates that the more unpredictable an area is, the more specific enablement is necessary in order to satisfy the statute. In the instant case, the instant claimed invention is highly unpredictable since one skilled in the art

cannot fully described genus, visualize or recognize the identity of the members of the genus, by structure, formula, or chemical name, of the claimed subject matter, as discussed above in *University of California v. Eli Lilly and Co.* Hence, in the absence of fully recognizing the identity of the members genus herein, one of skill in the art would be unable to fully predict possible physiological activities of any compounds having claimed functional properties in the method of the particular treatment herein.

Moreover, one of skill in the art would recognize that it is highly unpredictable in regard to therapeutic effects for the particular treatment herein, side effects, and especially serious toxicity that may be generated by drug-drug interactions when and/or after administering to a host (e.g., a human) the ***combination*** of any compounds represented by "one beta-mimetic", "at least one prostaglandin inhibitor", or " one calcium-blocking agent", which especially broadly encompass those known and **unknown** compounds of the recited functional compounds as of the instant filing date, as well as those **future** known compounds, that require additional or future research to establish or verify their usefulness.

See text book "Goodman & Gilman's The Pharmacological Basis of Therapeutics" regarding possible drug-drug interactions (9^{th} ed, 1996) page 51 in particular. This book teaches that "The frequency of significant beneficial or adverse drug interactions is unknown" (see the bottom of the left column of page 51) and that "Recognition of beneficial effects and recognition of and prevention of adverse drug interactions require a thorough knowledge of the intended and possible effects of drugs that are prescribed" and that "The most important adverse drug-drug interactions occur

with drugs that have serious toxicity and a low therapeutic index, such that relatively small changes in drug level can have significant adverse consequences" (see the right column of page 51) (emphases added). In the instant case, in the absence of fully recognizing the identity of the members genus herein, one of skill in the art would not be able to fully predict possible adverse drug-drug interactions occurring with many combinations of any compounds having claimed functional properties in the pharmaceutical compositions herein to be administered to a host. Thus, the teachings of the book clearly support that the instant claimed invention is highly unpredictable.

The presence or absence of working examples and the quantity of experimentation necessary:

Moreover, it is noted that the specification fails to provide working examples, i.e., testing results or data to demonstrate the instant combinations with different combinations to be administered to a host, for reducing any oxytocin-mediated action in a mammal.

Thus, the specification fails to provide **sufficient** support of the broad use of any compounds having those functions recited in the instant claims. As a result, necessitating one of skill to perform an exhaustive search and undue experimentation for the embodiments of any compounds having those functions recited in the instant claims suitable to practice the claimed invention.

As discussed above, *Genentech*, 108 F.3d at 1366, states that "a patent is not a hunting license. It is not a reward for search, but compensation for its successful

conclusion" and "[p]atent protection is granted in return for an enabling disclosure of an invention, not for vague intimations of general ideas that may or may not be workable".

Therefore, in view of the Wands factors, the case *University of California v. Eli Lilly and Co.* (CAFC, 1997) and *In re Fisher* (CCPA 1970) discussed above, to practice the claimed invention herein, a person of skill in the art would have to engage in *undue experimentation* to test all compounds encompassed in the instant claims and their combinations employed in the claimed method to be administered to a host, with no assurance of success.

Response to Argument

Applicant's arguments filed November 12, 2004 with respect to this rejection made under 35 U.S.C. 112, first paragraph, for lack of full scope of enablement of record in the previous Office Action have been fully considered but are not deemed persuasive as further discussed below.

Applicant asserts that "Applicants' specification provides non-limiting examples of particular beta-mimetics, prostaglandin inhibitors and calcium-blocking agents that can be used in combination with a thiazolidendione compotmd. See, e.g., the specification at pages 13 and 14" and that "[t]he fact that the use of tocolytic agents are known in the art is strong evidence that undue experimentation would not be required to also administer thiazolidendione in combination with tocolytic agents".

However, given their broadest reasonable interpretation during patent examination as noted in MPEP 2111, the instant claims are **not limited** to those particular known agents such as ritodrine, magnesium sulfate, indomethacin, and

nifedipine disclosed in the specification. On the contrary the instant claims read on administering to a patient the ***combination*** of any substances or compounds or agents represented by "one beta-mimetic", "at least one prostaglandin inhibitor", or " one calcium-blocking agent", and thiazolidinedion herein for reducing any oxytocin-mediated action in a mammal.

These functional recitations may reasonably encompass those known and **unknown** or **future known** compounds having the recited functions as of the instant filing date. Note that those **future known** compounds have not yet been discovered and/or made as of the instant filing date. Hence, those unknown or future known compounds encompassed by claim 1 herein **must** require to additional or future research to discover, establish, make and/or verify their usefulness. Therefore, as indicated in the previous Office Action, the skilled artisan has to exercise **undue experimentation** to practice the instant invention.

As noted in MPEP 2164.01, "Any analysis of whether a particular claim is supported by the disclosure in an application requires a determination of whether that disclosure, when filed, contained sufficient information regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention. The standard for determining whether the specification meets the enablement requirement was cast in the Supreme Court decision of Mineral Separation v. Hyde, 242 U.S. 261, 270 (1916) which postured the question: is the experimentation needed to practice the invention undue or unreasonable? That standard is still the one to be applied. In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

In this case, those Wands factors have been clearly discussed in the previous Office Action. Again, in the absence of fully recognizing the identity of the members genus herein, one of skill in the art would be unable to fully predict possible physiological activities of any compounds having claimed functional properties in the method of the particular treatment herein.

Moreover, one of skill in the art would recognize that it is highly unpredictable in regard to therapeutic effects for the particular treatment herein, side effects, and especially serious toxicity that may be generated by drug-drug interactions when and/or after administering to a host (e.g., a human) the ***combination*** encompassed by the claims herein. See text book “Goodman & Gilman’s The Pharmacological Basis of Therapeutics” regarding possible drug-drug interactions (9th ed, 1996) page 51 in particular.

Further, it is noted that the specification fails to provide working examples, i.e., testing results or data to demonstrate the instant combinations with different combinations to be administered to a host, for reducing any oxytocin-mediated action in a mammal.

Thus, the specification fails to provide clear and convincing evidence in sufficient support of the broad use of any compounds having those functions recited in the instant claims. As a result, necessitating one of skill to perform an exhaustive search and undue experimentation for the embodiments of any compounds having those functions recited in the instant claims suitable to practice the claimed invention, given the fact that the pharmaceutical art is unpredictable, requiring each embodiment to be individually

assessed for physiological activity. *In re Fisher*, 427 F.2d 833, 166 USPQ 18 (CCPA 1970). .

For the above stated reasons, said claims are properly rejected made under 35 U.S.C. 112, first paragraph, for lack of full scope of enablement.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 2-19 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention, for the same reasons of record stated in the Office Action dated May 19, 2004.

The recitation "a subject" renders these claims indefinite. The recitation "a subject" is not clearly defined in the claims or specification. One of ordinary skill in the art could not ascertain and interpret the metes and bounds of the patent protection desired as to what "a subject" would be, for example, that the term " subject " would be a single cell, any biological system, an animal, or a mammal or a human or any subject. Thus, one of ordinary skill in the art could not interpret encompassed thereby.

Claim 9 contains the abbreviation or trademark/trade name BRL49653. Where a trademark or trade or abbreviation name is used in a claim as a limitation to identify or describe a particular material or product, the claim does not comply with the requirements of 35 U.S.C. 112, second paragraph. See *Ex parte Simpson*, 218

USPQ 1020 (Bd. App. 1982). The claim scope is uncertain since the abbreviation or trademark or trade name cannot be used properly to identify any particular material or product. A abbreviation or trademark or trade name is used to identify a source of goods, and not the goods themselves. Thus, a abbreviation or trademark or trade name does not identify or describe the goods associated with the trademark or trade name. In the present case, the abbreviation trademark/trade name or abbreviation is used to identify/describe particular agent, accordingly, the identification/description is indefinite.

The recitation, "<u>a compound related to troglitazone</u>" in claim 9 render claim 9 indefinite. Hence, one of ordinary skill in the art could not ascertain and interpret the metes and bounds of the patent protection desired as to "a compound related to troglitazone" of various kinds of compounds since any significant structural variation to a compound would be reasonably expected to alter its properties, e.g., physiological effects and functions. Thus, it is unclear as to what "a compound related to troglitazone" of compounds herein would be encompassed thereby.

Response to Argument

Applicant's arguments filed November 12, 2004 with respect to this rejection made under 35 U.S.C. 112, second paragraph of record in the previous Office Action have been fully considered but are not deemed persuasive as further discussed below.

Applicant asserts that “Applicants' specification and claims provide non-limiting examples of “subjects" that are contemplated by the present invention. See, e.g., the specification at page 4, lines 21-23”. Contrary to Applicant’s assertion, the specification at page 4, lines 21-23, is not seen to clearly define what “subject" is. Thus, the instant

claims are clearly not limited to those particular examples described in the specification; exemplification is not an explicit definition.

Applicant is suggested to amend claim 9 by reciting the real name taught in the specification at page 15, line 30 for BRL49653.

Applicant argues that the specification defines "A compound related to troglitazone is one that is substantially similar to the chemical structure of troglitazone or can be derived from troglitazone" (see page 5, line 8-10). Applicant's argument is not found persuasive, since one of ordinary skill in the art could not ascertain and interpret the metes and bounds of the patent protection desired as to "a compound related to troglitazone" of various kinds of compounds having many possible substitutents, given the fact that any significant structural variation to a compound would be reasonably expected to alter its properties, e.g., physiological effects and functions. Thus, it is unclear as to what "a compound related to troglitazone" of compounds herein would be encompassed thereby.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 2-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Antonucci et al. (US 5457109, PTO-892) in view of Hanif et al. (PTO-892) and Soloff et al. and Fuchs et al. (PTO-1449 submitted May 20, 2002), for the same reasons of record stated in the Office Action dated May 19, 2004.

Antonucci et al. discloses that the particular thiazolidinedione compounds having formula I which covers and encompasses the instant particular thiazolidinedione such as, troglitazone (see its chemical name disclosed at col.15 lines 26-28), is useful in methods for the treatment of normal pregnant women or non-diabetic pregnant women <u>due to insulin resistance</u> and/or related risks (see col.3 and Example 1 at col.18 lines 35-46). Antonucci et al. also discloses various routes for administering the particular thiazolidinedione compound to a host (see col.18-24).

Antonucci et al. does not expressly disclose the employment of the particular thiazolidinedione compounds, in methods for reducing oxytocin-mediated action in a pregnant mammal such as the reduction of inducing labor and uterine cramps or contraction in a pregnant mammal, inducing milk letdown, and inducing prostaglandin release.

Hanif et al. teaches that "oxytocin confers a state of insulin resistance on the adipocyte, probably acting at a post-receptor site". Hanif et al. also teaches that "apparently, in the adipocyte, oxytocin acts via the same receptor as is present in uterine and breast smooth muscle and the metabolic actions of oxytocin are due to mechanisms in common (chem. mediators, phosphorylation-dephosphorylatio reactions) with the ones involved in the action of insulin. See abstract in particular.

Known functions of oxytocin (OT) include smooth muscle contraction during birth (see Soloff et al. 1989 and Fuchs et al. 1982), milk letdown during lactation (see Soloff et al. 1979) and prostaglandin release from endometrium/deciduas and the anmnion (see Hinko and Soloff et al. 1993).

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to employ the particular thiazolidinedione compounds, in methods for reducing oxytocin-mediated action in a pregnant mammal such as inducing labor and uterine cramps or contraction in a pregnant mammal, inducing milk letdown, and inducing prostaglandin release.

One having ordinary skill in the art at the time the invention was made would have been motivated to employ the particular thiazolidinedione compounds, in methods for reducing oxytocin-mediated action in a pregnant mammal such as the reduction of inducing labor and uterine cramps or contraction in a pregnant mammal, inducing milk letdown, and inducing prostaglandin release, because thiazolidinedione compounds are known to be useful in methods for the treatment of normal pregnant women or non-diabetic pregnant women due to insulin resistance and/or related risks according to Antonucci et al. It is also known that teaches that oxytocin confers a state of insulin resistance on the adipocyte, probably acting at a post-receptor site and in the adipocyte, oxytocin acts via the same receptor as is present in uterine and breast smooth muscle and the metabolic actions of oxytocin are due to mechanisms in common with the ones involved in the action of insulin according to Hanif et al. Moreover, oxytocin (OT) functions are known to include smooth muscle contraction during birth, milk letdown

during lactation and prostaglandin release from endometrium/deciduas and the anmnion according to the prior art.

Therefore, one of ordinary skill in the art would have reasonably expected that particular thiazolidinedione compounds, would have beneficial therapeutic effects and usefulness in methods for reducing oxytocin-induced action in a pregnant mammal such as inducing labor and uterine cramps or contraction in a pregnant mammal, inducing milk letdown, and inducing prostaglandin release, by common mechanisms with the ones involved in the action of insulin e.g., treating insulin resistant,as reducing or decreasing oxytocin-induced actions.

Claims 15-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Antonucci et al. (US 5457109, PTO-892) in view of Hanif et al. (PTO-892) and Soloff et al. and Fuchs et al. (PTO-1449 submitted May 20, 2002) further in view of Dullien (US 5,370,135, PTO-892), for the same reasons of record stated in the Office Action dated May 19, 2004.

The same disclosures of Antonucci et al. (US 5457109) in view of Hanif et al. and Soloff et al. and Fuchs et al. have been discussed in the 103(a) rejection set forth above.

The prior art does not expressly disclose the employment of a tocolytic agent in combination with thiazolidinedione in methods for reducing oxytocin-mediated action in a pregnant mammal such as inducing labor and uterine cramps or contraction in a pregnant mammal, inducing milk letdown, and inducing prostaglandin release.

Dullien teaches that a number of known tocolytic agents are known to be used for treating premature labor or pre-term labor by reducing uterine contractions (see col.1).

It would have been obvious to a person of ordinary skill in the art at the time the invention was made to employ a tocolytic agent in combination with thiazolidinedione in methods for reducing oxytocin-mediated action in a pregnant mammal such as inducing labor and uterine cramps or contraction in a pregnant mammal, inducing milk letdown, and inducing prostaglandin release.

One having ordinary skill in the art at the time the invention was made would have been motivated to a tocolytic agent in combination with thiazolidinedione in methods for reducing oxytocin-mediated action in a pregnant mammal such as inducing labor and uterine cramps or contraction in a pregnant mammal, inducing milk letdown, and inducing prostaglandin releasesince, since a number of known tocolytic agents are known to be used for treating premature labor or pre-term labor by reducing uterine contractions based on the teaching of Dullien.

Therefore, one of ordinary skill in the art would have reasonably expected that combining thiazolidinedione and a known tocolytic agent, both known useful for the same purpose, i.e., treating premature labor or pre-term labor by reducing uterine contractions, would improve the therapeutic effects for treating the same diseases, and/or would produce additive therapeutic effects in treating the same.

It has been held that it is prima facie obvious to combine two compositions each of which is taught by the prior art to be useful for same purpose in order to form third

composition that is to be used for very same purpose; idea of combining them flows logically from their having been individually taught in prior art. See *In re Kerkhoven*, 205 USPQ 1069, CCPA 1980.

Thus the claimed invention as a whole is clearly prima facie obvious over the combined teachings of the prior art.

Response to Argument

Applicant's arguments filed November 12, 2004 with respect to this rejection made under 35 U.S.C. 103(a) of record in the previous Office Action have been fully considered but are not deemed persuasive as to the nonobviousness of the claimed invention over the prior art as further discussed below.

Applicant asserts that "[t]here is no motivation to combine the cited references, and the Action has provided no evidence to the contrary" and that "[I]t appears that the action is equating an "obvious to try" rationale to support the obviousness rejection.

In contrast to applicant's assertions of the rejection is based upon an "obvious-to-try" standard; it is by now well understood that the ultimate conclusion of law that claimed subject matter as a whole would have been obvious under 35 USC 103 may at times properly be drawn from an inference of fact arising from prior art teachings which could be considered an inference that it would be "obvious to try" that which is claimed. In re O'Farrell, 853 F.2d 894, 7 USPQ 2d 1973 (Fed. Cir. 1988); Contour Saws Inc. v. Starrett Co., 444 F. 2d 433, 170 USPQ 433 (Ct.App. 1977); In re Marzocchi, 439 F. 2d

220, 169 USPQ 367 (CCPA 1977); In re Lindell, 385 F. 2d 435, 155 USPQ 521 (CCPA 1967).

Moreover, it must be recognized that any judgment on obviousness takes into account knowledge which was generally available and within the level of ordinary skill at the time the claimed invention was made. In this case, thiazolidinedione compounds are known to be useful in methods for the treatment of normal pregnant women or non-diabetic pregnant women due to insulin resistance and/or related risks according to Antonucci et al. It is also known that teaches that oxytocin confers a state of insulin resistance on the adipocyte, probably acting at a post-receptor site and in the adipocyte, oxytocin acts via the same receptor as is present in uterine and breast smooth muscle and the metabolic actions of oxytocin are due to mechanisms in common with the ones involved in the action of insulin according to Hanif et al. Moreover, oxytocin (OT) functions are known to include smooth muscle contraction during birth, milk letdown during lactation and prostaglandin release from endometrium/deciduas and the anmnion according to the prior art.

Therefore, one of ordinary skill in the art would have reasonably expected that particular thiazolidinedione compounds, would have beneficial therapeutic effects and usefulness in methods for reducing oxytocin-induced action in a pregnant mammal such as inducing labor and uterine cramps or contraction in a pregnant mammal, inducing milk letdown, and inducing prostaglandin release, by common mechanisms with the ones involved in the action of insulin e.g., treating insulin resistant, as reducing or decreasing oxytocin-induced actions, as pointed out in the previous Office Action.

Applicant also asserts that "the Antonucci et al. reference is non-analogous art" and "Antonucci et al. does not concern Applicants' field of endeavor and is not reasonably pertinent to reducing the induction of labor". One cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. In re Keller, 642 F.2d 413, 208 SPQ 871 (CCPA 1981); In re Merck & Co., Inc., 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). See MPEP 2145.

Therefore, as discussed above, the motivation provided by the combined teachings of the prior art to make the present invention is seen. The claimed invention is obvious in view of the prior art.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970);and, *In re Thorington,* 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 CFR 1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claim 2 is rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-3 of U.S. Patent No. 6,537,566.

Although the conflicting claims are not identical, they are not patentably distinct from each other because the patent is drawn to a method of treating uterine fibroids by administering the fibroid cell growth inhibitor, a thiazolidinedione (see claim 3 of the patent) which is the same active agent to be administered herein.

The claim of the instant application is drawn to a method for reducing oxytocin-mediated action in a subject. One having ordinary skill in the art at the time the invention was made would recognize that a method for reducing oxytocin-mediated action in a subject administering the thiazolidinedione compound would encompass a method of treating uterine fibroids also by administering the same thiazolidinedione compound. Thus these methods between in the patent and in the instant application are seen to substantially overlap.

Thus, the instant claim 2 is seen to be obvious over the claims 1-3 of U.S. Patent No. 6,537,566.

Applicant is requested to note that the obviousness-type double patenting rejection of record in the previous Office Action was intended to reject claim 2 not the cancelled claim 1, since the context of the rejection clearly indicates claim 2. The typographic error is regretted.

In view of the rejections to the pending claims set forth above, no claims are allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Jiang, whose telephone number is (571)272-0627. The examiner can normally be reached on Monday-Friday from 9:00 to 5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Sreenivasan Padmanabhan, Ph.D., can be reached on (571)272-0629. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

S. Anna Jiang, Ph.D.
Primary Examiner
Art Unit 1617
February 9, 2005

APPENDIX 3
Related Proceeding Appendix

None